ELEMENTARY BACTERIOLOGY

M. L. DHINGRA, M.D.

ELEMENTARY BACTERIOLOGY

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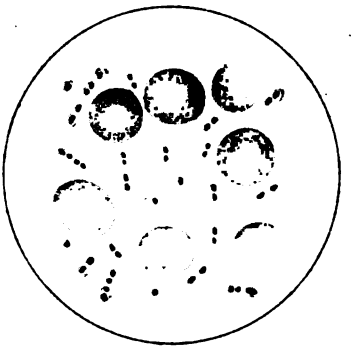

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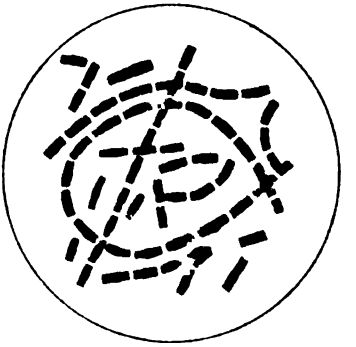
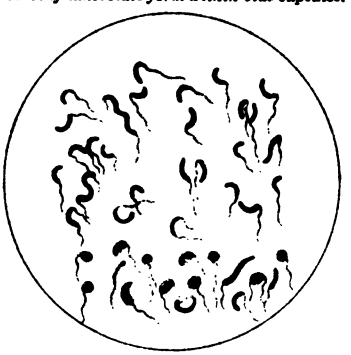


II. *B. Anthracis from culture—large blue bacilli containing red spores.*



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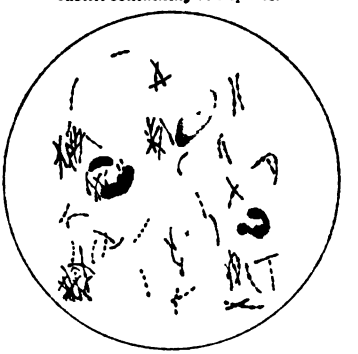

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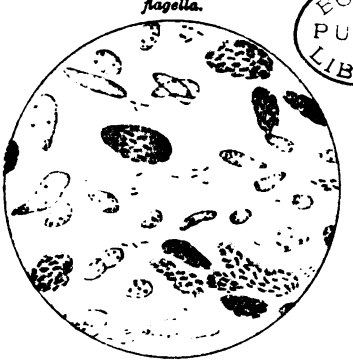

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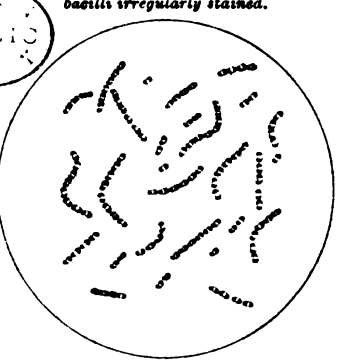

VI. *B. pestis from culture—polar staining.*

West, Newman chromo.

Figs. I.–IV. and VI. from Quain's 'Dictionary of Medicine.'
Fig. V. from Curtis' 'Essentials of Practical Bacteriology.'

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M. L. DHINGRA, M.B.

DIPLOMATE IN STATE MEDICINE

MEMBER OF THE SANITARY

WITH COLOURED PLATES AND ILLUSTRATIONS IN THE TEXT

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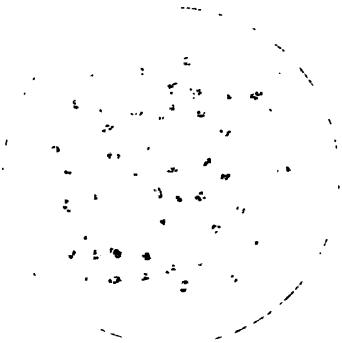

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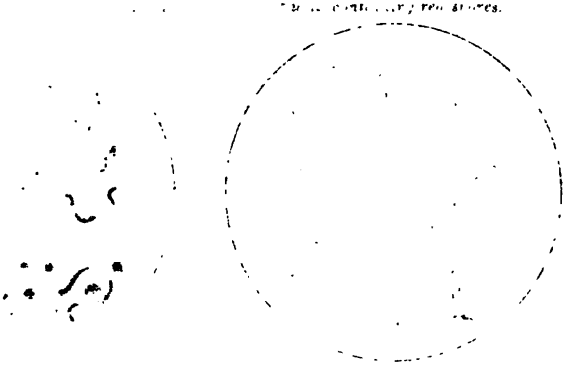

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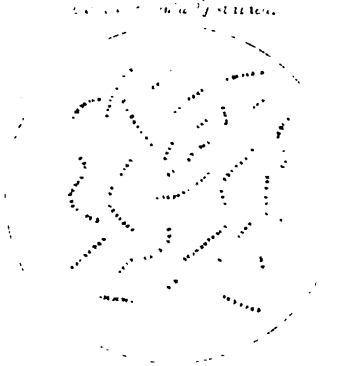

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BY

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MEMBER OF THE SANITARY INSTITUTE, ETC.

WITH COLOURED FRONTISPIECE
AND ILLUSTRATIONS IN THE TEXT

LONGMANS, GREEN, AND CO.
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NEW YORK AND BOMBAY
1903

TO THE MEMORY OF

R. D.

WHOSE CONSTANT SYMPATHY AND ENCOURAGEMENT

ENABLED ME TO WRITE THESE PAGES

I GRATEFULLY DEDICATE

THIS WORK

PREFACE.

AN attempt has been made in the following pages to represent, in a concise manner, the fundamental principles of Bacteriology. As will be seen from the contents, the material has been so selected and arranged as to meet with the ordinary requirements of Indian students and practitioners. For obvious reasons the practical portion of the subject has been treated in the briefest possible manner.

It may be necessary to add that in the preparation of this work the author has aimed, not so much to marshal facts as to point out principles and suggest problems. Thus, while the achievements of this science are fully acknowledged, its difficulties and limitations are also frankly recognised. This method of presenting a subject is not without its drawbacks, but has the great advantage of contributing to the development of that philosophical or scientific spirit which is at once the aim and object of true culture.

I gladly take the opportunity of expressing my

thanks to Mr. H. J. Curtis, F.R.C.S., and Dr. Alexander Crombie, C.B., for much kind advice and help throughout this work. I am also indebted to Prof. Calmette, of Lille, for many valuable suggestions on the subject of Snake-poisoning.

Lastly, I must acknowledge the extreme courtesy shown to me by the publishers on all occasions.

M. L. DHINGRA.

CLARENDON ROAD, W.,
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PART I.

CHAPTER I.

THE THEORY OF SPONTANEOUS GENERATION.

THERE can be no doubt that the question of the origin of organic life must have exercised as much fascination in the earliest ages as it does to-day. The attention of poets and philosophers was naturally arrested by this question, and they believed that the living always arose from the non-living.

The doctrine of spontaneous generation was an accepted article of faith with the writers of the Middle Ages, until an Italian chemist improved upon this by giving a recipe for creating mice! However, it was not till 1668 that Francesco Redi, by the simple device of covering a jar with wire-gauze, actually demonstrated that the maggots present in putrid meat did not arise *de novo*. In 1675 Leeuwenhoeck, "the father of microscopy," by the aid of a simple lens, discovered the presence of extremely small organisms in putrescent fluids. Later, the development of the compound microscope showed microbes everywhere, and a veritable germ-mania prevailed.

The question of the origin of these minute germs was thus once more brought to the front; but no real advance was made till the middle of the eighteenth century, when an English divine, Needham, instead of relying on mere assertions, conceived the happy idea of putting the matter

to the test of an experiment. He boiled infusions for several minutes in flasks, which were then hermetically sealed, and found that organisms rapidly developed in the contents. He concluded, therefore, that the germs originated spontaneously in the lifeless constituents of the liquid.

Needham's observations were combated by an Italian divine, Spallanzani, who claimed that in infusions enclosed in air-tight vessels, and boiled for a whole hour, no organisms appeared.

But Needham objected that Spallanzani, by the excessive heat which he employed, had so altered the air in the vessels, that it was no longer suitable for the development of life. To meet this objection, Schultz, in 1836, only half filled the flasks with the infusions, into which the external air (passed through bulbs containing sulphuric acid) was sucked daily. On examining the contents after several months, no organisms were detected; but they soon appeared when the flask was opened, and exposed to the air.

But objectors still urged that the treatment of the air, although not violent, had nevertheless altered its composition. Schröder modified Schultz's experiment by making the incoming air pass through cotton wool, instead of sulphuric acid. This treatment could not be called "violent," and yet the air completely lost the power of decomposition. These experiments certainly suggested that there was something in the air, capable of giving rise to organised beings.

Now, what is the nature of this *something?*

Before answering this question, it will be as well to pause at this stage, and to interpret some of the results thus far achieved. Perhaps the most instructive feature of this controversy is the progressive limitation of cases

of spontaneous generation. Beginning with the higher animals, it became more and more limited till mice and flies were gradually excluded. It is obvious that the cause of decomposition is not a gas, for that would not be excluded by filtration through cotton wool, but is something discontinuous, something *particulate*. Further, as these particles are destroyed by heat and sulphuric acid, they are probably of organic origin.

The doctrine of spontaneous generation was thus apparently lost, but the results obtained by the experimenters were by no means uniform, and, in many instances, previously boiled liquid underwent decomposition. In 1858 Pouchet appeared as an advocate of this theory, contending that organic molecules can be derived from previously living molecules. His well-conducted experiments and his zeal brought him many adherents, and spontaneous generation was once more in high favour.

Things were at this pass, when Pasteur undertook the study of this subject and, by a series of classical researches, demonstrated that it is possible to preserve any organic substance provided the heating be sufficiently prolonged and the external air be carefully excluded. He showed further that the atmospheric dust is the exclusive cause of life in organic infusions, and demonstrated this by an ingenious experiment. He boiled the liquid in a flask, the neck of which was drawn out in the form of an "S," and then left it for some time. The fluid did not decompose, although it was in contact with the external air, because the dust was arrested in the bend of the tube. But if the vessel were violently shaken, the dust was dislodged and, entering the fluid, rapidly brought about its decomposition. "And therefore," exclaimed Pasteur in the course of his memorable address, "I

could point to that liquid and say to you, I have taken my drop from the immensity of creation, and I have taken it full of the elements appropriated to the development of inferior beings. And I wait, I watch, I question it, begging it to recommence for me the beautiful spectacle of the first creation. But it is dumb, dumb since these experiments were begun several years ago; it is dumb because I have kept it from the only thing man cannot produce, from the germs which float in the air, from life, for life is a germ and a germ is life. Never will the doctrine of spontaneous generation recover from the mortal blow of this simple experiment."

Pasteur, however, was somewhat premature in his conclusion, as decomposition still occurred even with careful precautions. This was found to be due to the presence of "spores" or seeds, and, with the discovery of these resistant forms, the doctrine of spontaneous generation received its final death-blow. *Omne vivum e vivo* was thus shown to be true not only with regard to the higher beings, but of the lowest unicellular organisms as well.

This leads us naturally to the question: Whence came the ultimate and primary creature? How did life originate on our globe? The hypothesis of Lord Kelvin, that life was brought to the earth by shooting meteors from other planets, evades the question and only throws it one step further back. It would then be asked: How did life originate on these heavenly bodies? If not the result of a miracle, it must have been due to spontaneous generation. As a matter of fact, we have in the "nitrifying" (see p. 38) and other allied bacteria, some of the representatives of the earliest forms of life, which are ceaselessly at work in producing the living from the non-living materials. Evidently these organisms must

have played an all-important rôle in the evolution of life on our globe.

Strictly speaking, the experiments cited above merely show that spontaneous generation has not been proved experimentally. But it does not follow therefrom that spontaneous generation is impossible. Although the actual production of the living from the non-living substances cannot be imitated in the laboratory, yet there are certain considerations which show that such changes can take place. Scholl has demonstrated that ferments, which may be said to occupy an intermediate position between the lifeless proteids and the living cells, may be rendered less active by heat and rejuvenated by suitable treatment. Prof. Bose of Calcutta has recently shown that metals respond to electric and other stimuli much in the same way as the nerves of animals do. These results indirectly support the theory of spontaneous generation, for they tend to obliterate the line of demarcation between the organic and the inorganic, the living and the non-living.

This recognition of the hypothesis of spontaneous generation can do no harm. It is in complete harmony with the law of origin from ancestors, which, as we have already seen, is capable of universal application. And, although the experimental proof be wanting, the possibility of spontaneous generation must be frankly admitted, if bacteriology is to take its rank among the exact sciences.

CHAPTER II.

FERMENTATION.

OBSERVATIONS on the phenomena of fermentation are of great antiquity, and were first made by those who prepared the juices of sugary plants, *e.g.*, that of the grape. The agitation which the entire mass undergoes, and the continued production of gaseous bubbles which break on the surface, must have recalled to them the ebullition of liquids exposed to heat. It is to this striking analogy to the process of boiling that the word "fermentation" owes its derivation (*fervere* = to boil). The worship of Bacchus testifies to the fact that the ancients were acquainted with the properties of wine, and there is ample evidence to conclude that alcoholic fermentation was empirically known in the prehistoric epoch.

In the writings of alchemists in the Middle Ages the word "fermentation" is frequently used; but its application was too vague and comprehensive. Being unacquainted with elementary chemistry, every chemical action was to them a sort of fermentation. Some were, no doubt, struck with the fact that a small amount of leaven transforms into fresh leaven an enormous quantity of dough. The knowledge of this property of the transmission of a force to a large mass, without the original force being itself weakened in the process, naturally led them to seek for the "philosopher's stone".

Nothing of any consequence, however, was accomplished

till the end of the eighteenth century, when chemistry made a great stride under the genius of Lavoisier. Starting with the principle of the indestructibility of matter, he endeavoured to ascertain the chemical process involved in alcoholic fermentation.

In 1837 Schwann discovered the remarkable fact that fermentation is not a mere chemical molecular transformation, but a physiological process of the *living yeast cells*. The dependence of fermentation on living yeasts is shown by the facts that living cells are present in all fermenting fluids, and that fermentable substances do not ferment when the entrance of living yeast cells is prevented by heat or filtration through cotton wool. And further, the intensity of fermentation runs parallel with the development of the organisms in the fermenting fluid.

Liebig considered all fermentations as molecular movements, which a body in a state of chemical movement, *i.e.*, decomposition, transmits to other substances whose elements are not very firmly combined. He denied the all-important rôle of living organisms.

Pasteur overthrew this theory by a series of ingenious researches, and thereby laid the foundation of fermentation physiology. He, however, thought that fermentation was "life without air". But this is incorrect, as many fermentations occur in abundance of oxygen.

Admitting, then, that living cells are the cause of fermentation, let us proceed to inquire as to how this action is brought about.

According to one explanation, fermentation is due to the action of certain ferments or *enzymes*, which the organisms secrete, in common with other animal and vegetable cells. Buchner, by subjecting yeast cells to high pressure, has obtained a liquid substance which, without the intervention of living cells, is capable of

inducing alcoholic fermentation. But it is not certain that this pressure-extract is really an enzyme. Macfadyen, in repeating Buchner's experiment, found that the substance of crushed yeasts, of itself, contains alcohol, which shows that the actual fermentative changes take place in the interior of the cells. Besides, the numerous by-products formed in the fermentation process cannot be due to the action of enzymes, but rather to the metabolic activity of the cell-protoplasm.

The ammoniacal fermentation of urea, however, is undoubtedly due to an enzyme, which has recently been isolated from its organism—the *micrococcus ureæ*. But in this case, the process is one of simple hydrolysis, and no by-products are formed.

It is not suggested that enzymes do not play an important part in all fermentative processes. But their function is largely limited to the preparatory changes which they induce in the nutrient medium, rendering it more assimilable by the living protoplasm. Thus, the inversion of sugars and peptonisation of albumens is the result of the enzymes secreted by the organisms. But the actual fermentative changes are brought about in the protoplasm itself, and are the direct result of its metabolic activity.

We see, then, that the process of fermentation is intimately connected with the presence of the living organisms which consume the elements necessary for their growth, and cast off the residue as excretory products. The result is the degradation of the complex organic compounds and their rearrangement into simpler ones.

All organisms capable of exciting fermentation possess certain characteristics in common. Pasteur long ago ascertained that all organised ferments are:—

(1) Nitrogenous organic bodies.

(2) They are unstable.

(3) A relatively small quantity can produce great changes in the substance acted upon, especially if the metabolic products are removed as quickly as they are formed.

To these points must be added the *specificity* of ferments, that is to say, each ferment produces its particular fermentation and no other. Thus if we introduce simultaneously in the same saccharine medium, alcoholic, lactic, and butyric ferments, we see three distinct reactions side by side—one breaks up the sugar into alcohol and CO_2; the second converts it into lactic acid; and the third into butyric acid.

Apart from the nature of the organism, the products of fermentation will vary with the nature of the fermented body. Heat and moisture are necessary conditions for the fermentation process, but neither of them can give rise to it.

CHAPTER III.

FERMENTATION (*CONTINUED*).

THE process of fermentation is so important both from a scientific and a commercial standpoint that it is desirable to give some instances of its applications in industrial processes. It will then be seen how numerous are the products formed by bacterial agency, and how largely mankind is indebted to such organisms.

Alcoholic Fermentation.—When grapes are crushed the grape sugar is fermented—alcohol and CO_2 being formed.

To what is this change due?

It is caused by a torula or *yeast* naturally present on the skin of the grapes. The latter when crushed permit the organisms to operate on the juice, which disappears, or rather reappears as alcohol.

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$

Groups of bud — Mother-cell

FIG. 1.—Yeast Cells. [From Schenk's *Bacteriology*.]

Yeast cells are the most ubiquitous of all fungi. They are round or oval in form, and are made up of granular protoplasm, surrounded by a definite capsule. They reproduce by "budding".

When yeast cells are grown on an unfavourable medium, as gypsum block, they form spores within their capsule. This fact was taken advantage of by Hansen for the identification

of numerous varieties of yeasts; and he also pointed out that there were special organisms for special beers. By using pure yeasts a scientific accuracy is obtained, and nothing is left to chance. The results of Hansen's researches have completely revolutionised the brewing industry, and pure growths of yeast are now sent out from his laboratory to all parts of the world, different yeasts being employed for different beers.

It will be noticed that alcoholic fermentation is caused by yeast and not by bacteria proper. But although the latter are not so directly concerned, they are important in so far as they cause diseases of beers and wines.

Acetous Fermentation.—If we take some beer or weak solution of wine, and allow it to stand for a time in contact with air, it gradually turns sour owing to the conversion of alcohol into acetic acid. The change is essentially an oxidation of alcohol.

$$C_2H_6O + O_2 = C_2H_4O_2 + H_2O.$$

This oxidation can also be brought about by purely chemical means. Thus if alcohol be passed over spongy platinum, acetic acid results as before. But this method is impracticable on a large scale.

To produce acetic acid for commercial purposes, weakened alcohol is poured through a tall cylinder filled with wood shavings, the latter having been first inoculated with some warm vinegar. After a number of hours the resulting fluid is charged with acetic acid.

It has been stated that the process is a purely chemical one, and that it takes place without the intervention of bacteria. But this can hardly be so in this method, for the addition of vinegar is really the addition of living microbes—the *mycoderma aceti*. These grow upon the shavings, and oxidise the alcohol into acetic acid.

The organisms concerned in the process are of various species. They usually form chains of short or long threads, and each appears to act best under different conditions.

Lactic Acid Fermentation.—It is this fermentation which is commonly seen to occur in fresh milk, and any sample of sour milk may be relied upon to contain an abundance of *lactic acid organisms*. They curdle milk and produce lactic acid from milk sugar.

$$C_6H_{12}O_6 = 2C_3H_6O_3.$$

Fresh milk possesses a rich bacterial flora, but lactic bacteria find conditions in milk so favourable to growth, and they so completely outstrip the other organisms, that at the end of twenty-four hours they outnumber and even check the growth of all other forms. They are abundant in the atmosphere of cowsheds and dairies, and are, therefore, a considerable source of annoyance both to the salesman and the consumer. Fortunately lactic organisms never form spores, and thus can easily be destroyed by a moderate heat.

Lactic fermentation plays an important part in the preparation of fodder, in the manufacture of butter, and in the tanning of leather. The souring of wines and beers is likewise due to the activity of the lactic bacteria.

Butyric Fermentation is important historically, having been first studied by Pasteur, and having led to the discovery of the anærobes. It is caused by the *Bacillus butyricus* and the *Bacillus amylobacter*—two organisms possessing this characteristic, *viz.*, that they can only grow in the absence of oxygen. They occur plentifully in air, dust, and milk, and are also commonly met with in putrefactive processes.

In ordinary milk they are prevented from growing by

the *lactic acid bacteria* and the presence of air. But if milk be boiled they are uninjured (owing to spore formation) and produce butyric fermentation, especially if milk be left without access of air. They find their way into butter, and are the chief cause of the development of rancidity.

Indigo Fermentation.—Indigo is obtained from certain species of the Leguminosæ. The colouring matter does not exist ready formed in the plant, but is developed therefrom by the fermentation of a glucoside constituent, known as *indican.* The plants are cut down shortly before flowering time, immersed in water, and then allowed to ferment. The indican is first changed into indigo-white which remains in solution, but this is soon oxidised into indigo-blue and precipitated as an insoluble body.

Of the intimate nature of this process, we are as yet unaware. But there can be no doubt that it is a true fermentation induced by a special bacterium, which occurs on the leaves. When the leaves are sterilised, no fermentation follows, but the characteristic blue is at once seen if the specific organisms be added to the mass.

Commercial indigo contains, in addition to the blue, red, brown and other colouring matters in different proportions, so that the shade of colour is variable in different samples of indigo. To control this fermentation and thereby to produce at will any desired variety of pigment, is a problem well worth the study of modern bacteriologists.

Tobacco Fermentation.—Tobacco leaves after being dried are piled up in big heaps where they undergo a kind of fermentation. Within a short time the temperature rises, and when it reaches 130° F. the piles are taken down and rearranged. This is repeated six or seven times, and, at the

end, the tobacco is in a proper condition for the market. The changes produced in the process are a decrease in nicotine, disappearance of sugar, and the production of flavour.

It cannot be claimed that all these changes are due to bacterial activity, as physico-chemical processes must also play an important part. But as various flavours are developed during the fermentation process, it is very probable that they are due to the different types of fermentation which the tobacco undergoes.

Now, if the flavours are due to bacterial agency, it should be possible to modify them by altering the fermentative process. Experiments have recently been made with cultures of bacteria found in Havanna tobaccos, in artificially inoculating tobaccos grown elsewhere, in order to develop desired flavours; and the results are certainly encouraging.

Summary.—The foregoing illustrations suffice to indicate the usefulness of bacterial agency. It is obvious that without their beneficent aid we could have neither "bread and butter" nor "wine and cigar".

But it must not be supposed that bacteria invariably produce these desirable fermentations. On the other hand, they are frequently the cause of great trouble. Undesirable bacteria often gain access during the fermentation process, produce unpleasant flavours, and greatly lower the value of the product. This is constantly seen in the various industrial processes cited above.

The problem, then, is: How to control the fermentative process so that it may be possible to produce the desired product at will? An answer to this question has been partially furnished by Hansen, whose method of *pure cultures* of yeasts has already revolutionised the brewing

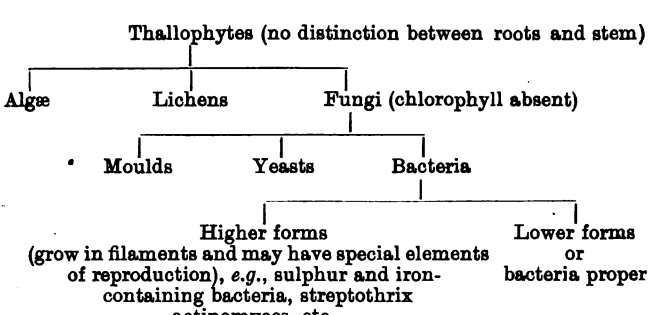
industry. The brewer now enjoys complete command over the process, there is less risk of "diseases," and more profit. The results in dairies are no less striking. Conn has recently isolated a bacillus, pure cultures of which give the desired flavour to butter. It is not improbable that the use of pure cultures in tobacco and indigo fermentations will lead to better results in the future.

The principle that in order to obtain certain desired species of plants its seeds should be sown free from the seeds of all other plants is well known to the students of horticulture; and the use of pure cultures in fermentative industries is but a further application of this principle. Pure cultures, however, cannot alter the chemical composition of the substance, for this depends on soil and climatic conditions. But even with this limitation they have an immense range of usefulness in the scientific working of fermentative industries.

CHAPTER IV.

THE MORPHOLOGY OF BACTERIA.

BACTERIA are the smallest and simplest of living creatures, and can only be seen with the aid of high magnification by the microscope. They are the lowest members of the vegetable kingdom, and differ from unicellular animal organisms in the fact that, unlike the latter, they are nourished through the cell wall by substances held in solution. They are also peculiar in their mode of growth, and in forming spores (or seeds). Their relation to the vegetable kingdom can be easily seen from the following table :—



A study of the above table enables us to formulate a definition of bacteria, which would be : *minute unicellular organisms multiplying by transverse fission, and containing no chlorophyll.*

In *structure* they resemble an ordinary cell with its protoplasm and a limiting membrane, but no nucleus has been demonstrated. The protoplasm consists of a structureless albuminous substance, which in its reaction to aniline dyes resembles the nuclei of ordinary cells. The living substance may be broken up by vacuoles; or it may contain iron, sulphur and various pigments.

The cell-membrane is composed of a substance allied to cellulose. It sometimes swells and gives rise to a gelatinous envelope, which cements together adjacent bacilli. A colony (*zooglœa*) is thus formed; but each cell remains distinct, and there is no attempt at a division of labour.

In *form* the bacteria are usually either spheres, rods or spirals; although atypical forms are not uncommon. In old cultures they readily undergo degeneration or "*involution*," and various irregular and distorted forms are produced.

When the bacterial cell is in the form of a sphere it is called a *coccus* or micrococcus. On the other hand, when the cell is not iso-diametrical, but exhibits a difference between length and breadth, it is a *bacillus*. If the rod is bent like a German comma it is called a *vibrio*. When the rod is longer and spirally twisted we get a *spirillum*; while numerous turns like those of a corkscrew give rise to a *spirochœte*.

The *mode of reproduction* is very characteristic. The cell elongates and then divides in the middle into two halves, each of which repeats the process. It is to be noted that these organisms are not generated, neither do they die. They merely divide. The protoplasm of the parent still exists in the daughter cells, and, barring accidents, is immortal.

If the daughter cells, instead of being detached, remain connected with one another, they give rise to character-

istic groupings. Thus, the cocci may be arranged in pairs (*diplococci*); or in clusters like a bunch of grapes (*staphylococci*); or in the form of a chain (*streptococci*). If the division in the case of cocci takes place in two directions we get *tetrads;* and if in three directions we have *sarcinæ*, a common content of diseased stomach.

Among bacilli, owing to the relation between the transverse and longitudinal diameters, there is room for a wider range of morphological characters. Thus they

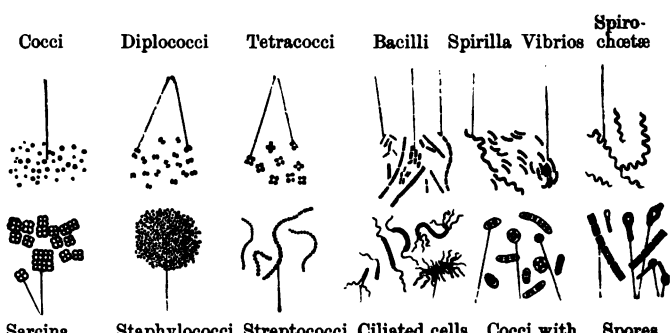


FIG. 2.—The Morphology of Bacteria.
[From Schenk's *Bacteriology*.]

differ not only in size and mode of grouping, but also in form.

The bacteria are so minute in *size* that a special standard of measurement has to be employed. This is the micro-millimetre, or $\frac{1}{1,000}$ of a millimetre, and is represented by the Greek letter μ. It is equivalent to $\frac{1}{25,000}$ of an inch.

Bacteria may vary in size from 0·3 μ to 30 μ. An average-sized coccus would not be more than 1 μ in diameter; so that 25,000 of such individuals placed in a row would make a chain one inch long. Even the comparatively large-sized anthrax bacillus is only 3 to 10 μ long and 1 to 1·2 μ

broad, so that its volume would have to be increased eight million times before it attained to the bulk of an ordinary cigarette.

Being devoid of chlorophyll, micro-organisms can only feed on organic matter, which is presented to them in a state in which it can be readily absorbed. The sources of food being, however, plentiful, the rate of their *multiplication* is simply enormous. Under favourable circumstances bacteria have been observed to divide in about half an hour, and it is calculated that in one day a single cholera germ would produce a progeny of sixteen hundred trillion. In nature, however, it is hardly likely that so great an increase ever takes place. Lack of food and improper physical conditions very often bring about a rapid diminution in their number.

Spore Formation.—Under the influence of conditions, some of which favour and others retard their growth, bacilli enter into a resting stage, and assume a shape endowed with great powers of resistance. The protoplasm shrinks from the cell membrane, and gathers into one or more rounded masses or *spores*. Each spore, when ripe, appears as a brilliant body surrounded by a highly resistant spore wall. The capsule finally bursts, and the spherical body is set free.

Like the seeds of higher plants, spores resist desiccation and heat, and may retain their vitality for months or years. They successfully resist a temperature of 100° C. for a short time; whereas ordinary bacteria succumb to a temperature of 60° C. They may be said to be the hardiest form of living substance known.

When the conditions are again favourable for germination the membrane bursts, and the contents grow out as a bacillus.

Pathogenic organisms do not form spores so long as

they are enclosed within the body; but they may do so in their saprophytic or external phase of existence. The question whether a certain organism forms spores is an important one from the hygienic standpoint, for it gives us a measure of the resistance of the organism, and therefore of the risk of infection. Non-sporing organisms are easily destroyed, and their infection readily controlled.

Sporulation must not be confused with reproduction. The former is a guise for the preservation of the species; the latter is concerned with its propagation. A spore gives rise to a single individual; but an organism divides into two.

Locomotion.—Bacteria, like all solid particles when suspended in fluids, exhibit the so-called Brownian movements, which consist of oscillations, without any actual movement of translation. This phenomenon is not to be confused with the true locomotion seen in the case of many bacilli or vibriones. Thus if a young culture of a motile organism (*e.g.*, the cholera germ) be examined under the microscope in a so-called "hanging-drop" preparation,[1] the organisms are seen to be dancing and shooting across the field with an amazing rapidity.

The organs of locomotion are slender whip-like appendages called *cilia* or *flagella*, which, like oars, propel bacteria through water. The disposition of flagella is characteristic. There may be numerous processes distributed over the whole surface (as in the typhoid bacillus); or there may be only one or two at the poles (as in the cholera vibrio); or there may be numerous polar flagella (as in the *spirillum rubrum*).

Variability of Bacteria.—Micro-organisms, like all the lower members of animal and vegetable kingdoms, dis-

[1] See p. 131.

play considerable variations in their characters. Indeed, at one time, it was maintained that there was no constancy among bacteria, and that the same species might exhibit diverse forms and properties. Therefore it was argued that there were no different species among them.

That micro-organisms should be so excessively polymorphic could hardly be a matter of surprise, considering how profoundly they are influenced by environment. If the conditions of existence fluctuate—as must frequently be the case in nature—one of two things may happen. Either the organisms change their form and function and adjust themselves to the new conditions, or they may fail to adapt themselves, and so perish. In the first case, the modifications of biological characters may persist through numerous generations, and lead to the production of new varieties. When we remember that there are already about 500 varieties of potato, although it was only introduced into Europe about two or three centuries ago, we need not be surprised at the countless varieties of bacteria which have been cultivated since the beginning of the earth, and can give rise to fifty generations in the course of twenty-four hours.

And yet it must be confessed that the extraordinary variability of bacteria is very confusing. The same species may give rise to a rod, a thread or a spherical form. The *bacillus prodigiosus* produces red spots on starchy substances; but when grown at a higher temperature brings about lactic fermentation, without the production of pigment. The organism of pneumonia, *i.e.*, the *pneumococcus*, causes indigo fermentation; but is also pathogenic for man. And the remarkable variability of the cholera vibrio is well known (see p. 77).

But notwithstanding these variations there is no doubt that under identical conditions the properties of bacteria

will remain constant. It is no more possible to convert one species into another than it is among higher plants. Pus cocci, however cultivated, can never be changed into cholera vibrios, and all attempts to convert the *B. coli* into *B. typhosus* have failed. Again, it is not always possible by selecting the external conditions to produce other species at will, or to suppress a single biological character in any species.

We conclude, then, that bacteria are divisible into species and genera just in the same way as the higher organisms. It is true that they present considerable departures from the form that is deemed "typical". But just as a cinchona tree may normally show considerable variations both in the amount and variety of alkaloids it contains, a germ may also show atypical characters and be still within the boundaries of the species. Indeed, it may be affirmed that under all circumstances there exists a well-marked form for each species, which represents for the latter the maximum of its growth, the climax of its well-being.

Classification.—But although distinct species of bacteria do exist, their differentiation from one another is a matter of considerable difficulty. Hence it is that the classification of bacteria is in such an unsatisfactory state. Organisms have been divided, according to the characteristic effects they produce, into *chromogenic* (colour producing), *photogenic* (light producing), *ærogenic* (gas producing), etc. But as the same micro-organism may produce any of these effects it is obvious that a biological classification is impossible. The division of bacteria into *saprophytes* (which grow on dead matter) and *parasites* (which grow in living tissues) is based on the assumption that organic matter is their necessary pabulum, and can no longer be entertained. For the discovery of *nitrogen-*

fixing bacteria (p. 42) has shown us that certain forms of life can dispense with organic and inorganic matter, and live simply on atmospheric nitrogen. To meet this difficulty Fischer has suggested the division of all bacteria into three groups as follows :—

1. *Prototrophic.*—These require no organic food, and include nitrifying, nitrogen-fixing, sulphur- and iron-bacteria.
2. *Metatrophic.*—These require organic matter for their growth, and comprise *facultative parasites* (*i.e.*, saprophytes capable of taking on a parasitic existence), and most bacteria.
3. *Paratrophic* bacteria occur only in living tissues (*obligatory parasites*).

While this mode of classification has its value, the most practical division is that which is based on morphological characters. As has already been stated, the division of bacteria into spheres, rods, and spiral forms is fairly satisfactory, and will be found amply sufficient for our purpose.

CHAPTER V.

THE GENERAL BIOLOGY OF BACTERIA.

ACCORDING to the generally accepted hypothesis of the origin of the earth, it is obvious that the first formed bacteria must have existed under conditions totally different from those which prevail to-day. The atmosphere must then have been poorer in oxygen and light, but of a higher temperature; and there must also have been a notable lack of organic food-stuffs. It is, therefore, interesting to find certain species which still retain their primitive ancestral characters, and can only grow without light, oxygen or organic matter; and others again which thrive only at the boiling temperature. It is important, then, to bear in mind that although bacteria in general can grow when supplied with suitable food and temperature, yet their precise requirements admit of the greatest variations.

A.—CONDITIONS OF GROWTH.

1. *Nutrient Media.*—Like all higher plants, bacteria require for their food materials whence they obtain the elements of which they are made up. *Carbon* is derived from carbohydrates, and *nitrogen* from albuminoids, or more rarely from inorganic compounds. In addition to these sources of nitrogen and carbon, all media must contain *salts* and an abundance of *water*. A neutral or faintly

alkaline reaction is generally indispensable. The media usually employed in the laboratory are bouillon, gelatine, agar, blood serum, etc.

Bacteria, however, are exceedingly fastidious in their demands. Thus, while some are easily satisfied with ammonium salts, others refuse to grow unless cultivated in blood serum, and still others refuse to grow at all in any of our artificial media. Again, the range of their requirements is so narrow that trivial differences in the composition of food materials may favour the growth of one organism in preference to the other. Nägeli found that in a neutral saccharine fluid containing bacteria, yeasts and moulds, only the first flourished causing lactic fermentation; but the addition of ½ per cent. of tartaric acid caused the growth of yeasts; while the addition of 4 to 5 per cent. of the same acid brought about the development of moulds.

It is interesting to note in this connection a remarkable property which some micro-organisms possess, *i.e.*, the power of *selection*, whereby they can discriminate even between extremely similar substances. Mannite and dulcite are two such substances, and the difference between them is so slight that both are represented by the same chemical formula. But there is a class of organisms which can distinguish between them more easily than the chemist; and if grown in their mixture will attack mannite, leaving dulcite untouched. To take another remarkable illustration: if yeast be added to a solution of fructose, which consists of right-handed and left-handed lævulose, it only attacks the left-handed molecules, leaving the former severely alone. This is probably due to the fact that the yeast organisms, from centuries of growth, have had deeply impressed upon them the capacity of acting upon a particular form of lævulose; but they cannot so

act on the right-handed lævulose, which is a mere laboratory product, and has never been encountered in nature.

2. *Oxygen.*—While many organisms grow only in the presence of air (*obligatory ærobes*), others do so only when oxygen is completely excluded (*obligatory anærobes*). But the great majority of bacteria, although thriving best in the presence of oxygen, can also grow when its supply is more or less cut off (*facultative anærobes*). This is true of almost all pathogenic forms, which are thus uninjured in the body cavity, where the oxygen is scanty or absent. But it is probable that in this situation their vegetative capacity is diminished, and the formation of toxic metabolic products increased. The exclusion of oxygen suspends chromogenesis in nearly all colour-forming organisms, and destroys the phosphorescence of all photogenic bacteria.

3. *Temperature.*—In their relation to temperature, bacteria present wide differences. Thus, while most water bacteria can grow at the freezing temperature, many of the saprophytic forms flourish at the boiling temperature.

Between these cold-loving and heat-loving bacteria lie almost all parasites which grow best at the blood-heat. Although most organisms grow within a definite range of temperature, yet the maximum development takes place at a certain point called the "*optimum*". The range of temperature necessarily varies with each class of bacterium, and is well brought out in the following table:—

	Minimum.	Optimum.	Maximum.
B. phosphorescens	0° C.	20° C.	38° C.
B. subtilis	6°	30°	50°
B. anthracis	14°	37°	45°
B. tuberculosis	30°	37°	42°
B. thermophilus	42° C.	63-70° C.	72° C.

It will be observed that while *B. subtilis* ("hay bacillus") can grow within very wide limits, the *B. tuberculosis*, like all strict parasites, has the narrowest range of all. It is also apparent that a necessary condition for pathogenesis is the approximation of the optimum temperature to blood-heat. Where such an approximation does not obtain the animal does not "take" the disease, *i.e.*, it is "immune". It is *partly* for this reason that man is insusceptible to the hay bacillus, and fowls are immune to anthrax. When the latter are immersed in cold water their temperature is brought down, and made to correspond with the optimum of *B. anthracis*, with the result that they speedily succumb to the disease.

Thermophilic bacteria are commonly met with in the fermentation of hay, cotton waste, tobacco, etc. Their optimum reaches the temperature of coagulation of most proteids. It is probable that their protoplasm is different from that of ordinary cells.

Cold arrests the growth of organisms, but does not destroy their vitality. Most bacteria withstand a temperature of several degrees below 0°; and yet at this temperature the ordinary life processes must cease. Under such conditions bacteria are neither living nor dead—they pass into a "*third state*," or a condition of suspended animation.

Heat, on the other hand, has a decidedly injurious action on the vitality of bacteria. If the optimum temperature be raised by 5° to 10° their power of growth and virulence are reduced, but not altogether lost. But if the maximum temperature is exceeded death follows owing to the coagulation of protoplasm. A temperature of 60° C. for ten minutes, or 70° C. for five minutes, is sufficient to kill most sporeless bacteria.

To destroy spores the medium is exposed to live steam

for fifteen minutes on three successive days, and during the intervals kept at 25° C. to 30° C. to allow spores to vegetate (*intermittent or fractional sterilisation*).

4. *Light.*—Direct sunlight checks the growth of all bacterial forms, and under favourable conditions may be bactericidal in its effects. Even when the light is not very intense, or when the exposure is too short, attenuation of the virus may result. If the action of heat be excluded by first carrying the light through a layer of water, no alterations in these results follow, showing that the germicidal action is due to light rays alone. The rays that are most effective are those of the highest refrangibility, *i.e.*, the blue and violet rays of the spectrum.

In considering these results we must remember that they are obtained in the laboratory, and under conditions which would rarely occur in nature. For practical disinfection, therefore, it would be most unsafe to rely on sunlight alone. But by introducing plenty of light and air into our dwellings we may attenuate the vitality of the germs, and thereby, to a certain extent, safeguard ourselves against infection.

5. *Association of Bacteria.*—Although for purposes of study pure cultures of bacteria are essential, we must not forget that in nature they often occur in combination. Their precise relations to each other can hardly be imitated in the laboratory, but recent investigations have thrown an interesting light on this question.

When several species are associated in the same culture one may take precedence and the others may grow later (*metabiosis*); or two or more species may develop simultaneously, and co-operate for their mutual advantage (*symbiosis*); or the growth of one species may prevent the development of another (*antibiosis*).

Metabiosis is well seen in the fermentation of "grape

must," where, although a number of organisms exist side by side, yeasts develop first, then the vinegar organisms, and lastly the putrefying bacteria.

Symbiosis, however, is more important, and may be illustrated by numerous examples. Thus, some bacteria cannot play their ordinary rôle without the aid of others, *e.g.*, the organisms of tetanus and of suppuration. Again, the virulence of certain organisms may be heightened by means of association, *e.g.*, diphtheria bacillus and streptococci. And the symbiotic relationship of legumes with the bacteria of root-nodules, is an even more beautiful illustration, and will be discussed in a subsequent chapter.

As regards the antagonism of bacteria, it has been found that *bacillus pyogenes fœtidus* prevents the growth of *spirillum choleræ;* and Emmerich has pointed out that animals injected with anthrax bacilli may be saved by subsequent infection with streptococcus pyogenes. According to Hankin, *micrococcus Ghadialli* destroys typhoid and colon bacilli; and he has therefore suggested the use of this coccus to purify water polluted with typhoid stools.

It is thus apparent that bacterial growth is modified not only by environment, but also by association with other species. Therefore, when a pure culture of a species is obtained, and doomed to a separate existence, certain alterations are necessarily induced in the biological characters of the species concerned. A closer study of this question is most desirable, not only for the sake of confirming our notions about the life-history of bacteria, but also to show how far a species can be associated with another so as to give rise to the development of powers which would otherwise remain unobserved and unutilised.

B.—The Vital Phenomena of Bacteria.

As is the case with all living cells, the amount of energy derived from the combustion of food-stuffs is not wholly expended in the building up of new protoplasm, but is, in part, applied to the production of diverse phenomena. For the sake of convenience they may be classified as follows :—

1. *Optical.*—Several species of bacteria have been isolated from sea-water which, when cultivated in suitable media, are markedly phosphorescent. The faint glow on decaying fish is also caused by the presence of these "*photogenic bacteria*". The phosphorescence is a vital phenomenon of the organism, and is therefore lessened by anything that interferes with its vitality. The death of the organism, or the exclusion of oxygen, quenches the light at once, while a more abundant supply of oxygen increases it.

Phosphorescence is absent in perfectly smooth water, and is best seen at the crest of the waves, where the oxygen is most plentiful.

2. *Thermic.*—The rise in temperature so frequently observed in the decomposition of organic materials, *e.g.*, hay, manure, tobacco, is no doubt due to bacterial agency. Sometimes the temperature may be so high as to cause "*spontaneous combustion*". It appears that these results are brought about by the growth of "*thermophilic bacteria*".

3. *Mechanical.*—If a drop of stagnant water be examined under the microscope it will be observed that bacteria tend to crowd round particles of organic matter to which they are mysteriously attracted (*chemiotaxis*). This is not the result of an instinct for food, but of the chemical nature of the substance employed as stimulant. The

chemiotaxis is said to be *positive* when the living cell approaches, and *negative* when it recedes from the chemical substance. Asparagin, peptone, and oxygen are strongly chemiotactic, but free acids and alkalies negatively so. Alcohol is despised by bacteria.

Chemiotaxis is not confined to the bacterial cell, but is also exhibited by leucocytes. It is in virtue of this property that the latter move towards the focus of production of toxins, and try to envelop the invading bacteria. If, however, the leucocytes are repelled, the infective agents grow unchecked, and death may follow as a consequence.

4. *Chemical.*—In order to render nutrient media more suitable for their absorption, bacteria resort to a peculiar device. They secrete ferments of various kinds, *e.g.*, proteolytic (transforming albumin into soluble substances), diastatic (converting starch into sugar), emulsifying, coagulating or fat-splitting. The action of these ferments, and the metabolism of the bacterial cells, give rise to various phenomena, the more important of which may be arranged in four great groups.

(i.) *Fermentation.*

(ii.) *Putrefaction*, or "putrid fermentation," is similar to fermentation, except that instead of occurring in carbohydrates, it takes place in dead *nitrogenous* material. The albumin is first peptonised, and then further broken up into such substances as carbon-dioxide, sulphuretted hydrogen, hydrogen, nitrogen, methane, butyric acid, indol, skatol, etc.

Sometimes the innocuous albumin is changed into distinct animal alkaloids called *ptomaines*. These may be formed outside the body, and become a source of danger when ingested with food. Poisoning with ice-cream or meat is frequently due to the presence of these substances.

The malodorous products of putrefaction are chiefly produced by anærobic bacteria, and are consequently most pronounced in situations where the supply of oxygen is limited or cut off. When a dead body is exposed to the air the anærobes migrate from the intestine, which is their normal habitat, and invade the tissues in all directions. They are, no doubt, favoured in their growth by the simultaneous presence (*symbiosis*) of the ærobes, which, while carrying on the surface decomposition, consume the oxygen and create an (anærobic) atmosphere so favourable to the continued development of the anærobes.

The organisms concerned in putrefaction are mostly *facultative anærobes*, of which the following are best known: *Proteus vulgaris*, *proteus Zenkeri*, *bacillus fluorescens liquefaciens*, *bacillus coli communis*, *bacillus saprogenes*.

(iii.) *Chromogenesis.*—An historical interest is attached to this property, as it was observed so long ago as the Middle Ages. The moist consecrated wafers left on the church altar were found the next day to be covered with little blood-red drops. This was supposed to foreshadow some dire calamity, and much capital was generally made out of this "miracle". It is now known, however, that this colour is produced by the growth of a common organism—the *bacillus prodigiosus*. It grows best at 25° C. on boiled rice or potato, and produces a red pigment which is insoluble in water, but soluble in alcohol. Owing to the ready production of pigment, the organism is frequently employed in various bacteriological investigations. It is also suitable for the study of the mutability of species. By repeated cultivations on faintly acid media the cells lose their globular shape, and become actively motile rods or threads. Again, by growing the bacillus at 38° C. a variety may be obtained which no longer produces pigment.

In addition to red, various other colours may be formed by bacterial activity. Thus, violet colouring matter is formed by *B. violaceus;* green or blue by *B. pyocyaneus* or *B. fluorescens;* and yellow by *staphylococcus pyogenes aureus.* A yellow coloration is also produced by *sulphur-bacteria,* which decompose H_2S, and store up the sulphur granules in their substance. Similarly, *iron-bacteria* oxidise the iron carbonates, and the oxide thus formed is deposited in the bacterial cell, causing it to assume a reddish-brown colour.

The pigment formation is best seen on solid media grown in the presence of air, away from sunlight, and at a low temperature. Chromogenic bacteria are frequently associated with putrefactive processes, and give rise to beautiful rainbow-like tints in putrescent substances.

(iv.) *Toxin production.*—Like many higher plants (*e.g.*, abrus precatorius, ricinus communis) bacteria secrete poisonous substances, which represent the specific action of the corresponding organism. They will, however, be more conveniently dealt with at a later stage.

CHAPTER VI.

THE PHENOMENA OF PUTREFACTION.

It may generally be said that the function of bacteria is to return all organised beings to earth. Every form of dead organic matter is immediately brought under their influence, and broken up into its elementary constituents. It is for this reason that fallen trees and dead bodies are cleared away from the ground, and make room for the succeeding generation. Thus far the microbes are our best friends.

But unfortunately their activities proceed further, for in the search for food they often prey upon living beings. When microbes attack living tissues they produce the manifestations of disease; but when the dead organic matter is attacked we have the phenomena of *putrefaction* and decay. At present, however, we are only concerned with the latter.

Bacteria as Scavengers.—If we examine a fallen tree in a forest it will be observed that it remains apparently unaltered for some time. Then it gets softened into a friable substance, which finally crumbles into a brownish powder and is incorporated with the soil. How are these changes brought about? The hard woody substance is first softened by various fungi which grow into it; next wood-eating insects appear; and finally bacteria complete the decomposition.

A similar dissolution is observed in the case of a dead

animal, but with this difference, that the agency here is chiefly bacterial.

The importance of these cleansing processes can hardly be over-estimated. Without bacteria the surface of the earth would be encumbered with dead organic matter, and there would be no room for vegetable and animal life. Microbes are the important agents of universal hygiene. They clear away the remains of all that has had life. Without them life would be impossible, for death would be incomplete.

The Nitrogen Cycle.—But the microbes do not merely scavenge and cleanse the surface of the earth. They do much more—they elaborate this material for further use. It is well known that plants are constantly being eaten by animals, and the latter in turn again by plants. But dead animals cannot be assimilated by plants unless they are brought into a proper condition by bacterial agency. Were it not so, owing to the constant loss of food-stuffs from the soil, vegetation would cease, and even animal life would become impossible.

It must not, however, be supposed that any new material is created by the micro-organisms concerned. It is the same food which is used over and over again, first by plants, then by animals, and then again by plants. This circulation of matter lies at the root of the continuation of our food supply, and, as we shall see presently, is largely brought about by the activity of microbes. A closer study of this question is pertinent to our present inquiry, not only on account of the profound interest of the subject itself, but also because it throws interesting sidelights on the behaviour and general biology of bacteria.

Plants obtain their food partly from the atmosphere and partly from the soil. Of the food-stuffs obtained from

the latter, the most important are the *nitrates* and ammonium salts. Under the influence of the sun's rays these are built up into *proteids*, which enter into the animal body when the plants are consumed. In this situation a portion of the nitrogenous food becomes partially broken down, and is daily excreted in the form of urea; while another portion remains behind locked up in complex organic compounds, and is only liberated on the death and dissolution of the animal.

Now comes the influence of the putrefactive bacteria, which attack all the excreted urea and dead organic matter. Various products result, but the most important is *ammonia.*

But even ammonia cannot be utilised by plants. Before this is rendered possible it must be oxidised into *nitrates.* This is accomplished by the agency of *nitrifying bacteria.*

That "nitrification" does occur in soils can be easily shown by analysing, at intervals, a definite quantity of soil, when an increase of nitrates will be observed after a few weeks. Now, if one portion of the soil is sterilised and the rest not so treated, the latter alone shows an increase in nitrates, showing that nitrification is a biological process, due to the presence of living matter in the soil.

These organisms refuse to grow on gelatine or other nitrogenous media, and hence the difficulty of the early experimenters. Winogradsky, however, hit on the ingenious device of using gelatinous silica, and thereby succeeded in isolating the organisms. Further investigations have shown that nitrification is the result of two distinct species of microbes: one of these (*nitrosobacteria*) oxidises ammonia into nitrous acid; and the other (*nitrobacteria*) converts nitrous into nitric acid, but is incapable of attacking ammonium carbonates.

Nitrifying bacteria do not need any organic matter for

their growth; indeed, its presence is even harmful to them. In this respect they are strikingly different from all other living organisms. They mineralise organic nitrogen, and present it to the plants in the form of nitrates, and thus form the last necessary link in the chain which binds the animal to the vegetable kingdom.

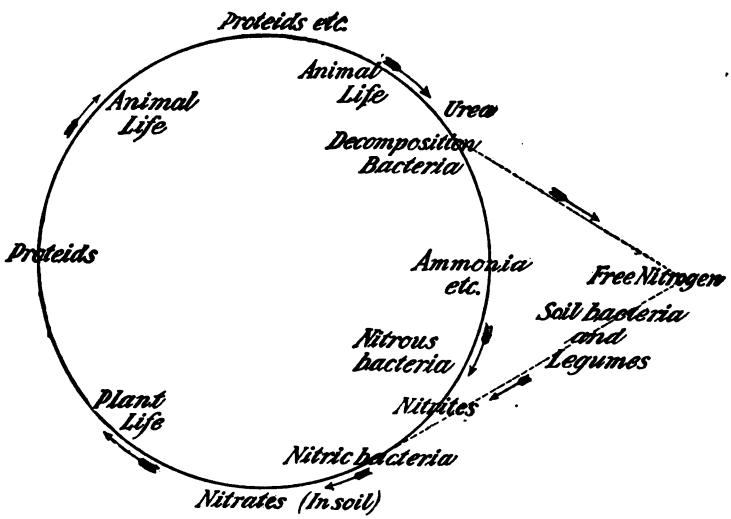


FIG. 3.—The Nitrogen Cycle.

Loss of Nitrogen.—Sometimes the process of nitrification is reversed, and nitrates are reduced to nitrites, and even still further to ammonia or free nitrogen. This is the result of the activity of the so-called "*denitrifying bacteria*". These organisms are commonly met with in fresh manure, and this explains why it is so wasteful to apply manure to land which has had a dressing of nitrates.

In addition to denitrification, a certain portion of nitrogen is constantly lost in the ordinary putrefactive processes. Nor are these the only sources of nitrogenous loss. Cremation and the discharge of sewage into rivers may be mentioned as other causes of this waste.

Reclaiming lost Nitrogen.—Since nitrogen is constantly taken out from the soil by vegetation, and a portion of it

FIG. 4.—A Leguminous Plant (Vetch) showing Root Tubercles.

is as constantly lost, it becomes necessary to inquire as to how vegetation has gone on for centuries, notwithstanding this loss.

As a matter of fact, the lost nitrogen is reclaimed by bacterial agency, which appears to act in two ways. In the first place, it is found that in most cultivated soils there are certain species of bacteria which are capable of fixing free nitrogen. This fixation, however, is not brought

about by a single species, but by the combined activity of two or more species acting together. Thus, *clostridium Pasteurianum* (a species allied to the butyric organism) possesses this power in a notable degree; but it can only so act if protected from oxygen by a zone of anærobic bacteria, which consume this gas and allow the clostridium to grow anærobically.

FIG. 5.—A Leguminous Plant (Vetch) without Root Tubercles.

The second method, however, is of greater importance, and affords a more beautiful instance of symbiosis. If the seeds of any leguminous plant, such as pea or bean, be grown into a soil containing all the necessary food-stuffs except nitrogen, it will soon begin to germinate. At first the growth takes place in the usual manner, and no apparent difference is observed, for the young plant feeds upon the store of nutrient substances contained in its cotyledons.

But as soon as this store is exhausted the growth becomes less vigorous, and finally ceases owing to the lack of food (nitrogen-hunger stage). All other plants (*e.g.*, wheat, etc.) suffer similarly under identical conditions. But while these plants continue in this stage, and finally die away, the legumes soon recover, throw up a luxuriant foliage, and produce a good crop of seeds. And what is still more extraordinary, they contain as much nitrogen as those plants brought up on nitrogenous manure. It follows from this that peas can, and do, assimilate nitrogen from the atmosphere.

Now, if a young legume be carefully pulled up from the soil, little swellings on the roots can be easily seen by the naked eye. It is known that only such plants as develop tubercles can increase the amount of nitrogen in their tissues, and further that the amount of nitrogen fixation is roughly proportional to the development of tubercles.

The tubercles contain bacteria in various stages of growth, and each kind of plant has an organism special to itself. These bacteria, which exist in the interstices of most soils, are attracted to the root hairs of a young legume, and by their active growth contribute to the formation of nodules.

We see, then, that the absorption of atmospheric nitrogen is connected with the development of tubercles, which latter are produced by the action of bacteria on roots. But neither bacteria nor plant can act alone—they must grow together (symbiotically) before nitrogen can be fixed.

When the plant dies the fixed nitrogen locked up in its tissues is brought under the influence of putrefactive bacteria, and nitrates are reformed. In this manner some of the lost nitrogen is brought back to the soil in the form of nitrates, and so commences the cycle afresh.

Summary.—The food cycle is now complete. Starting from the soil, we have followed the transference of matter successively through plants, animals, bacteria, and back again to the soil. It is interesting to note the remarkable division of labour among soil bacteria, by which complex organic compounds are first broken up and then elaborated into nitrates. The loss of nitrogen which occurs during this process is prevented by the specialisation of function of certain bacteria. These organisms bring the errant nitrogen back to the soil, and thereby tend to balance the input and output of the soil food.

And so the food-stuffs ceaselessly move on in this eternal cycle. And as long as the sun yields the necessary energy there is no reason why life should ever come to a standstill. But, as we have just seen, the continuation of food supply is largely dependent upon these ubiquitous bacteria. These "subtle artisans of nature" are constantly at work breaking up and rebuilding materials, and thus form the last link in the cyclical course of matter.

Nor does their importance end here, for they are of immense benefit to us in several other ways. And first let us look at the very disposition of soil bacteria. In the upper layers of the earth they are constantly oxidising off the organic matter and keeping the soil in a pure state. Lower down are anærobic organisms, which still further disintegrate the products filtered from above. Still lower down (at about twelve feet from the surface) no organisms are present, because the altered organic matter contains no nutriment for their growth.

The importance of these facts in relation to *water supply* is self-evident. This explains why superficial wells are generally impure, and deep wells pure. The sanitary engineer brings these organisms into his service,

and utilises them for the purification of our water supplies. He makes them grow on the surface of sand filters, so that the latter become not only mechanical, but biological filters as well. The bacterial purification of sewage is also brought about by the action of putrefactive bacteria.

Soil bacteria, however, play a more useful part in *agriculture*. From a study of their life-history we can deduce certain facts which are well worth the study of the scientific agriculturist. Since nitrification needs a great supply of oxygen, it is important that the soil should be well stirred, so as to admit air. Further, by the proper alternation of leguminous plants (*e.g.*, peas), with those which cannot fix nitrogen but simply use it (*e.g.*, wheat), it is possible to maintain the fertility of the soil, so far as it concerns nitrogen. Again, the advance of agricultural bacteriology has rendered it possible to replace artificial manures by the use of pure cultures of nitrogen-fixing bacilli. These, when introduced into the soil with the seed, are found to increase the produce in some cases. But the results, on the whole, are not satisfactory. This is probably due to the fact that "*nitragin*" (under which name cultures of nodule bacteria are sold in the market) contains only one variety of the organism, which is not equally useful for every species of legumes. Perhaps a better method would be the inoculation of a barren soil with leguminous earth, *i.e.*, a soil in which legumes flourish, and in which, therefore, many forms of nitrogen-fixing bacteria would be present. The subject, however, requires further elucidation.

CHAPTER VII.

ANTISEPTICS AND DISINFECTANTS.

It often becomes desirable to get rid of bacteria from certain media. A simple removal often suffices; as when air is passed through cotton wool, or when water is filtered through a porcelain filter. But in most cases this is not possible, and the destruction of the organisms becomes imperative. Fire is the best of germicides, but owing to its obvious disadvantage recourse is often had to various chemical agents. Those substances which retard the development of organisms without actually destroying them are called *antiseptics*. The complete destruction of their vitality is brought about by *disinfectants* or germicides. It, therefore, follows that all disinfectants are also antiseptics; but all antiseptics are not germicides.[1] The division between antiseptics and disinfectants, although convenient, is purely arbitrary. The same substance in different degrees of concentration may act now as disinfectant, now as antiseptic. Thus, 1:200 of carbolic acid kills; and 1:400 of the same acid restrains the bacillus of typhoid fever.

The method of determining the activity of a germicidal agent is comparatively simple. Organisms possessing a high degree of resistance (preferably anthrax spores) are first dried on sterile silk threads, and then introduced into

[1] It must be pointed out, however, that the term "antiseptic" is frequently used in the sense of "germicide".

a solution of the agent for varying lengths of time. By subsequent inoculations of nutrient media, it is noted whether the organisms have been destroyed or not.

It must be clearly understood, however, that the germicidal powers of an agent vary with the number and resistance of bacteria, the nature of the associated material, and many other conditions. Therefore the statement that a certain substance is disinfectant in a certain proportion is valueless, unless we are informed of the conditions under which its germicidal powers have been determined.

It would obviously be impossible to detail the numerous substances which have been employed as bactericidal agents, but the following are those most commonly used in practical disinfection :—

Acids.—Bacteria are more susceptible to the action of mineral acids, than to that of vegetable acids. It is for this reason that cholera vibrios, for example, are more readily destroyed by the hydrochloric acid in the stomach, than by the acids of fruits.

Potassium permanganate, in order to be of any value, must be used in sufficient quantity to destroy both the infective agent and its medium. According to Koch, as much as 5 per cent. of the salt is required to destroy anthrax spores in one day.

Ferrous sulphate is an antiseptic of moderate powers, largely used for the disinfection of privies.

Sulphur dioxide is extensively used for the disinfection of houses, but can hardly be called efficient. It is more effective in association with moisture, than in the anhydrous state. Unfortunately it bleaches vegetable colours, attacks iron, and injures cloth and leather.

Formaldehyde possesses none of the drawbacks of sulphur dioxide, and, being more potent in its action, has largely replaced this gas as a practical disinfectant. It

can be used either as a vapour, for which there are numerous forms of apparatus on the market, or as a solution. A 40 per cent. solution of formaldehyde is called "*formalin*". Formaldehyde is fatal to most bacteria in solutions of 1:1,000; and can be safely used for the disinfection of rooms.

Mercuric chloride, in the proportion of 1:1,000, may be relied upon to kill spores, provided, of course, no albumin is present. Plague bacilli are killed by two minutes' exposure to 1:3,000. Corrosive sublimate forms insoluble compounds with albuminoids, and then loses its bactericidal powers. This reaction is prevented by adding some sodium chloride, which forms, with the sublimate, a double salt soluble in water.

Zinc mercuric cyanide is one of the most powerful germicides known. A solution of 1:40,000 is sufficient to destroy the vitality of anthrax bacilli.

Carbolic acid, in a 5 per cent. solution, is recommended by Koch for the disinfection of cholera stools. Pus cocci are destroyed by a solution of 1:125 after two hours' exposure. Oily solutions of carbolic acid are useless, because the oil cannot penetrate into the organisms. On the other hand, the addition of hydrochloric acid decidedly increases the germicidal powers of carbolic acid.

In crude carbolic acid, besides phenol, cresol is present in large quantities, and is said to have greater disinfecting powers than phenol. Cresols, however, are not soluble in water, and various devices have been employed to secure their presence in solutions. Cresol is easily soluble in soap solutions; and advantage is taken of this fact in the preparation of various disinfectants which are advertised under the names of *Jeyes' fluid*, *Izal*, *Lysol*, etc. They are all slightly superior to carbolic acid in actual germicidal value.

As the chemical disinfectants are usually employed in aqueous solutions, investigations have been undertaken with regard to the nature of solutions, and they have led to considerable modifications in our views about disinfectants. According to the modern *theory of dissociation*, in a solution of a salt some of the molecules of the dissolved substance remain unaltered, while others are broken up (dissociated) into electropositive and electronegative constituents—into their *ions*. Now, many of the physical properties of a solution depend upon ionization, and it is certain that the toxicity of a salt also varies with its degree of dissociation. Thus, cyanide of mercury, which is very slightly dissociated, is a less efficient germicide than mercuric chloride. Indeed, the sublimate is the most electrically active, that is to say, the most toxic, of all other mercurial salts. To express the matter in more general terms, solutions of mercurial salts are the more active the more mercury they contain, not by atomic weight, but in the form of ions.

But the relation between dissociation and the disinfecting powers becomes even more marked when we compare the same salt in different degrees of dissociation. It is known that in a solution of corrosive sublimate ($HgCl_2$) the proportion of Cl ions to unchanged $HgCl_2$ molecules is constant. Now, if we add to this the strongly active solution of common salt (NaCl) more Cl ions are liberated, and the proportion of dissolved molecules to unaltered molecules is disturbed. The result is that the number of Cl ions derived from $HgCl_2$ is reduced, its degree of dissociation diminished, and some of the free Hg and Cl ions are built up again into complete molecules. It has been proved experimentally that as the degree of dissociation is lessened so is the toxicity reduced.

From this it follows that in the disinfection of fluids

containing sodium chloride a larger quantity of the sublimate must be added than would otherwise be required. The presence of albumin will demand a still further addition of the mercurial salt, because some of it will be precipitated in the insoluble compound with proteids. In the case of media rich in albumin (*e.g.*, fæces, sputum), the whole of the mercury may be precipitated, and its germicidal action entirely lost.

Besides the nature of the solvent, the temperature of the solution has an important influence on the degree of dissociation, that is to say, on the germicidal powers of the agent employed. Again, the knowledge that the action of germicides is chemical, and due to their combination with the albumin of bacteria, is apt to lessen still further our confidence in the permanence of their action.

It would serve no useful purpose to give a list of all the antiseptics, and the proportions in which they are most efficient. Such figures, obtained experimentally, have at best a theoretical interest, and may even be misleading. As has already been observed, there are a number of conditions which influence the germicidal powers of agents, and these must be carefully taken into consideration in the choice of a disinfectant. In the light of these facts the absurdity of sprinkling carbolic powders, and of pouring a deodorant down the gutter becomes apparent. It must be realised that the creation of a rival smell is no criterion of safety.

Klein has recently endeavoured to ascertain the value of various disinfectants by a more direct method. For this purpose he infected wood, cloth, and wall-paper with cultures of anthrax, tubercle, and cholera organisms, and then exposed them to the action of various germicides for twenty-four hours. It was found that carbolic acid,

corrosive sublimate, and sulphurous acid were the most efficacious, whereas Condy's fluid (1-53 to the pint) was the least reliable. Gaseous formalin, with only five hours' exposure, sufficed to destroy all organisms except when tubercle bacilli were present on the wood and cloth.

CHAPTER VIII.

THE PRESERVATION OF FOOD-STUFFS.

No better illustration could be furnished of the advantages of bacteriological study than the subject we are about to consider. This important industry owes its foundation to a Parisian, named Appert. While the scientists were discussing the theory of spontaneous generation, this enterprising confectioner perceived the possibilities of Spallanzani's experiment (p. 4), and perfected his process for preserving meat, vegetables, etc. To this end he exposed these substances to the temperature of boiling water for some time, and succeeded in obtaining the desired result. The modern methods of food preservation are essentially modifications of Appert's process. The use of borax, formalin, etc., as food preservatives is to be condemned, on account of their injurious effect on digestive enzymes. For it must be remembered that the problem which lies at the root of food preservation is this: how to destroy or suspend the vitality of micro-organisms, without at the same time lowering the nutrient value of the medium? The answer to such a question will necessarily vary with the nature of the food, and its bacteria flora; and, therefore, different methods are employed for different foods.

Milk, when secreted from the gland, is practically germ-free, but by the time it has entered the milk-pail it is extremely rich in bacterial contents. These come from

all sources: from the vessel, hands of the milker, air of the cowshed, and the dirt which adheres to the body of the cow. Of the non-pathogenic germs the lactic organisms are commonly found in milk, where they grow so rapidly as to check the growth of all other species. They may even prevent the growth of pathogenic forms, and would be most effective in preventing infection, were it not for the fact that milk is consumed long before this occurs. As is well known, milk is a frequent carrier of the infections of tuberculosis, cholera, and scarlet fever; and there is no doubt that various diarrhœal disorders are due to the bacteria of the "milk dirt".

A short *boiling* suffices to kill lactic and most pathogenic germs. But there are other milk bacteria which form spores, and these are not destroyed by the comparatively low temperature of ebullition. A boiled milk, therefore, may be rich in these bacteria, and yet remain unaltered to the naked eye. If, however, such a milk be consumed by a young infant, the spores develop into organisms, which rapidly decompose the milk and give rise to various gastro-intestinal disorders.

But this is not the only objection to boiling, for, as is well known, it alters the flavour and nutritive value of milk. The fat loses its emulsified condition separating out its cream, and the albuminoids are converted into a form very difficult of digestion.

Sterilisation by means of superheated steam has been tried to destroy spores, but without much success. It is found that this method is by no means efficient, as some spores still escape destruction.

A much better method is that of "*pasteurisation*," whereby milk is kept at 70° C. for fifteen minutes, and then *rapidly cooled*. The rationale of this process is simple. The high temperature is evidently below the

boiling point, and yet above the thermal death point of lactic and pathogenic germs. The spores and germs not so killed are checked in their growth by the rapid cooling, which is an important part of the process.

Pasteurised milk cannot be distinguished from fresh milk, which it closely resembles in flavour and nutritive value. It can be preserved for long periods, and is not so liable to give rise to diarrhœal diseases.

Pasteurisation has also been resorted to in the case of wines, cream, etc., and with marked success.

Meat.—It has already been remarked that the putrefaction of dead animals is initiated by the saprophytes of the alimentary canal, which migrate from their normal habitat and invade the tissues in all directions. If, therefore, the bowels of the animal be completely excised as soon as it is slaughtered, it would be possible to delay the decomposition for some time. This is precisely what the butcher does every day; but without understanding the rationale of his method.

To prevent further putrefaction, the disembowelled carcass is placed in a refrigerating chamber, and in this frozen state can be transported to long distances. This method of "*cold storage*" is largely employed in Australia, and has proved eminently satisfactory to the public and capitalist alike.

The *smoking* of meat is also a reliable means of preserving it. The smoke of heated wood chips is rich in phenol and creasote, which are deposited on the flesh and prevent putrefaction.

But the best method of preserving meat consists in *heating* it to a temperature sufficient to destroy all bacteria and their spores, and then hermetically sealing the vessels. This is essentially the process used by Appert long ago, and has led to the growth of a gigantic industry.

Eggs.—The contents of fresh-laid eggs are not always germ-free, as bacteria are known to enter the oviduct and infect the albumin before the shell is deposited. Again, the shell is porous, and allows bacteria and oxygen to pass through it. This gas is essential for the development of the embryo, and also for the organisms already contained in the egg.

The methods of preservation aim at making the shell impervious to the passage of bacteria and oxygen. This is accomplished by coating them with vaselin, or "water glass," a material composed of sodium and potassium silicates.

Vegetables and Fruits.—Bacteria require a preponderance of water for their growth, and they cease to grow in substances in which this element is lacking. It is for this reason that fruits and vegetables dried by the sun's rays or artificial heat can be preserved for comparatively long periods.

In the case of substances like green peas, which are apt to lose their flavour by desiccation, recourse is had to Appert's process.

In the preparation of jams and marmalades, the fruits are boiled, sugar added, and the whole carefully packed in sterile glass jars. Sugar in itself is an excellent food, and not only exalts the nutritive value of these substances, but at the same time plasmolyses [1] the bacteria, so that they are no longer capable of setting up decomposition. But in order to be effective it must be used in very strong solutions, otherwise it is apt to undergo alcoholic fermentation.

[1] By "*plasmolysis*" is meant a condition of the cell in which its protoplasm shrinks from the cell wall and aggregates into little masses.

PART II.

CHAPTER IX.

BACTERIA IN DISEASE.

Before considering the relations of bacteria to disease it would be as well to refer briefly to the organisms which are normally parasitic in man. They are present on the skin, in the respiratory passages, and in the digestive canal. Of these by far the largest number are met with in the intestines. Formerly it was supposed that bacteria were essential to normal digestion; but it is now recognised that their presence is by no means of any advantage to the host. On the other hand, they may be distinctly harmful, for they are liable to multiply whenever the defensive forces of the organism are diminished. There can be no doubt that many forms of headache and anæmia are due to *auto-intoxication* from the poisonous secretions of these microbes. Cirrhosis of the liver has been produced in animals by the aid of acetic and butyric acids, which are the normal products of our intestinal bacteria. It may also be that these organisms, by devitalising the mucous membrane, prepare the way for the cholera germs which are thus enabled to gain a foothold in the intestines.

The microbic theory of disease, although formulated by Henle in 1840, has only been demonstrated within the last few decades. Pasteur's researches on butyric fermentation led him to note the marked resemblance between the processes of fermentation and disease, and

he asserted that the organisms of fermentation were analogous to those described by Davaine in anthrax. Klebs in 1871 furnished the direct proof of the bacterial origin of disease by his studies on wound infections; and later researches have amply confirmed the microbic nature of infectious diseases.

But how do the microbes produce the lesions and symptoms of disease? Micro-organisms, although multiplying in the tissues of the host, are usually too insignificant in size to produce any appreciable result by their mere numbers. It appears, however, that this mechanical rôle is played in anthrax (as also in other septicæmic infections), where organisms fill up the lumen of capillaries, and thereby derange the normal metabolism of the affected parts. But in most cases the phenomena of disease are produced by the secretion of *toxins* or poisonous products, which enter into combination with the constituents of the body cells. In fact, it may generally be said that the bacterial infection is of the nature of intoxication. For although the multiplication of the organisms in certain tissues (alimentary canal, tonsils, etc.) is necessary to regulate the supply of toxin, the specific symptoms of the disease are produced by the latter alone. This can be easily demonstrated by injecting a germ-free culture, say, of the diphtheria bacillus (obtained by passing a broth culture of this organism through a Chamberland filter), into a susceptible animal, when the characteristic paralytic phenomena of the disease are instantly reproduced. But the local inflammatory lesions cannot be produced in this manner, which shows that some toxins are manufactured in the living tissues alone. As a matter of fact, pathogenic germs form a series of substances of varying degrees of toxicity, and thereby contribute to the complexity of the symptoms of infectious diseases. Thus,

the secondary anæmias which occur in the course of pneumonia, typhoid, etc., are due to the destructive action of certain of their toxins on the red-blood corpuscles of the host. But, although the bacterial products are of a complex nature, the specific symptoms are always produced by specific poisons.

The toxins may be soluble (*extracellular*), as in *B. diphtheriæ* and *B. tetani;* or they may be insoluble and contained in the bacterial cell (*intracellular*), as in the cholera and typhoid organisms. The intracellular toxins, also called "bacterioproteins," are common to many bacteria; and when injected into animals produce fever and inflammatory symptoms. According to Buchner, there is no hard and fast line between the intra- and extracellular toxins; and he regards both of them as closely associated with the cell protoplasm. For, just as in alcoholic fermentation, the yeast cells themselves contain alcohol, it may be that the toxins are first produced in the bacterial cells, and subsequently excreted into the surrounding medium.

It is, however, impossible to say whether the extracellular toxins are actually excreted, or are produced by the bacteria acting on the chemistry of the nutrient medium. Toxins have not been isolated in the pure state, and their precise nature is not known. Roux regards them as enzymes. According to most authorities, however, they are either proteids, or linked on to proteid molecules, and are not unlike certain vegetable and animal poisons, as ricin, abrin, snake venom,[1] etc. All these bodies are sometimes called *toxalbumins*.

The lesions produced in disease may be situated in the vicinity of bacteria, or at a distance. The former are

[1] See Appendix B, p. 138.

caused by the direct action of the toxins; and the latter by their absorption into the systemic circulation. The structural changes thus induced cause various functional disturbances, the so-called clinical symptoms.

It must be clearly understood that what is called a "disease" is not a specific entity, but a series of morbid manifestations, which, for convenience, have been arranged into groups. The classification of diseases was made long before the advent of bacteriology; and thus it may or may not correspond with the division based upon the germ theory. Pneumonia, for instance, is a distinct disease, and yet it may be caused by a variety of organisms. Again we know that the organism of pneumonia, besides producing this effect, may also give rise to œdema, suppuration or septicæmia.

On the other hand, many diseases are due to a single organism, which may truly be called specific. Anthrax, for example, is always caused by the *B. anthracis;* and this organism produces no other disease besides anthrax. Diphtheria is another notable example of a specific disease; but the same cannot be said of septicæmia or pneumonia. Koch has laid down that every specific pathogenic germ must fulfil the following conditions:—

(1) It must be constantly present in the diseased tissues, and must have special relations with the tissue changes.

(2) It must be capable of being cultivated on artificial media.

(3) The pure cultures thus obtained must reproduce the disease when inoculated into fresh animals.

The researches of the last few years have brought out the interesting fact that every pathogenic germ is really a member of a family of organisms possessing closely allied characters. This discovery does not invalidate the

doctrine of the *specificity of disease*, but is interesting from the evolutionary point of view, for it serves to remind us that all our parasites have arisen from saprophytes, by the process of natural selection. The organisms which simulate pathogenic forms are usually called "*pseudo-bacteria*". This term, however, is apt to be misleading, and will only exceptionally be used in the following pages.

CHAPTER X.

SUPPURATION.

EXPERIMENTALLY, suppuration may be produced by various chemical means, as, for example, by the subcutaneous injection of silver nitrate or oil of turpentine. But pus, as met with clinically, is usually formed by the action of certain bacteria, the most important of which are the *staphylococcus pyogenes aureus* and the *streptococcus pyogenes*. Several other varieties are also commonly present, as, for instance, *staphylococcus pyogenes albus* and *citreus*, and *bacillus pyocyaneus* (the organism of green pus). It may also be mentioned that the organisms of gonorrhœa, pneumonia, anthrax, etc., are frequently concerned in pus formation.

Staphylococcus Pyogenes Aureus.—This organism, which is normally present on the skin and in the respiratory mucous membrane, is the most common cause of suppurative processes in general. It can be obtained almost in pure cultures from boils, carbuncles, and the pus of osteomyelitis.

Under the microscope the organisms are observed as spherical cells forming groups or *clusters* of various sizes. They multiply rapidly at 20° C. in milk, broth, and other nutrient media. In "stab cultures" [1] the gelatine is liquefied in the form of a pouch, the bottom of which contains

[1] For methods of cultivation, staining, etc., see Appendix A, p. 129.

the characteristic *golden yellow pigment.* Upon the surface of agar, spherical colonies are formed which acquire an orange tint. Milk is rapidly coagulated, with the formation of lactic and butyric acids.

It has been shown that a weakened culture of the "golden staphylococcus" suddenly becomes virulent on the addition of a certain amount of grape sugar. This probably explains why boils are so frequently observed among those engaged in sugar refining. The carbuncles

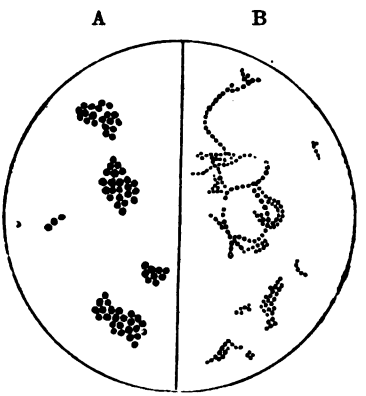


FIG. 6.—A, Staphylococcus Pyogenes Aureus. B, Streptococcus Pyogenes.
[From Curtis's *Essentials of Practical Bacteriology.*]

associated with glycosuria are, however, due to the lowered vitality of the tissues, which can no longer prevent the development of the pyogenic cocci normally present on the skin.

The pyogenic properties of this micrococcus can be easily demonstrated by rubbing a pure culture on the skin; or by subcutaneous injection in a rabbit or a guinea-pig. In fatal cases the most characteristic changes are observed in the kidneys, the capillaries of which are

plugged up with the micrococci. Metastatic abscesses may also be found.

Streptococcus Pyogenes.—This organism is found in acute abscesses, puerperal fever, ulcerative endocarditis, and (associated with the specific bacilli) in the diphtheritic false membrane, and, in fact, everywhere where there is suppuration. It is also frequently met with in the mouth and nose of healthy individuals. There is reason to believe that the so-called *streptococcus erysipelatis* is not a distinct species, but merely a variety of the streptococcus pyogenes.

Under the microscope the organism is seen to consist of spherical cocci arranged in pairs or in the form of a *chain.* In gelatine stab cultures numerous small white colonies are formed *without any liquefaction* of the medium. In blood serum the colonies readily appear in the form of minute white dots. Milk is slowly coagulated.

Injected subcutaneously, the streptococcus may set up a localised erysipelatous inflammation, the organisms being found in the lymphatic vessels and spaces at the spreading margin of the inflamed area. The subcutaneous tissue elements are not peptonised and broken down into pus, as in the case of the staphylococci. Should the cocci find their way into the cellular tissue, a cellulitis is produced, and if they enter into the circulation a septicæmia or pyæmia may follow.

The nature of the toxins secreted by the organism is not known. But horses have been successfully immunised against infection by repeated injections of sublethal doses of the living virus. The blood serum of these animals constitutes the *anti-streptococcus serum;* and has been extensively employed in those infections which are due to the *streptococcus pyogenes.*

Oriental Sore.—This is the name applied to a circumscribed inflammation of the skin that occurs endemically in Delhi, Lahore, Bagdad, and other places in the Eastern hemisphere. It begins as a papule, which sooner or later breaks down into an ulcer, and finally cicatrises. It is auto-inoculable; and can, moreover, be communicated to lower animals by the inoculation of discharges from the surface of the ulcer. The specific germ has not yet been isolated; but recent researches show that it is of the nature of *streptococcus*. As a rule second attacks do not occur.

CHAPTER XI.

GONORRHŒA.

It may be taken as established that the *gonococcus of Neisser* is the essential cause of gonorrhœa. When the gonorrhœal pus is stained with methylene blue, and examined under the microscope, the specific organisms are seen to be kidney-shaped, or like coffee beans. They

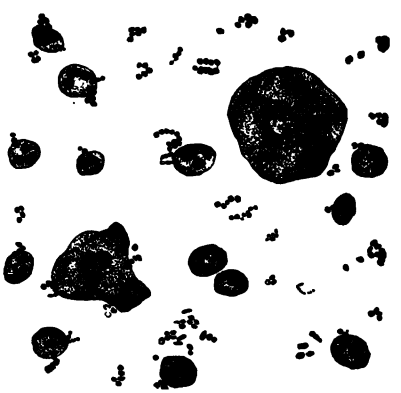


Fig. 7.—Gonococci from the Urethral Secretion.
[From Schenk's *Bacteriology*.]

are usually arranged in pairs, with the concave surfaces adjacent to each other. An important feature is the frequent presence of the cocci in the *interior* of the pus cells, where they may be found in considerable numbers. Another important point is that the organisms are not

stained by Gram's method, *i.e.*, they are decolorised by alcohol, after having been treated with an aniline dye, and the iodine solution employed in Gram's method of staining (p. 133).

Unlike other diplococci which may be present in the pus, the gonococcus refuses to grow on ordinary media, unless human serum is present at the same time. Blood-agar, or a mixture of fluid agar with albuminous urine, gives satisfactory results.

The gonococcus has only a slight resistance, the specific pus being rendered innocuous by exposure to 60° C. for ten minutes.

The ordinary laboratory animals are immune to gonorrhœa; but the disease can be reproduced in man by inoculating the healthy urethra with the gonorrhœal pus.

CHAPTER XII.

PNEUMONIA.

THE micro-organism, which is the essential cause of acute lobar pneumonia, was discovered by Sternberg, and fully studied by Fraenkel. It is variously known as the *diplococcus pneumoniæ*, *pneumococcus*, and *streptococcus lanceolatus* (*Frontispiece*, Fig. I.). This organism is highly pleomorphic, and its morphological characters vary according to the source from which it is derived. Thus, in the blood and exudates from infected animals the individual cocci are *lanceolate* or like a grain of wheat, possess a well-defined capsule, and are usually arranged in pairs (*diplococci*). But in cultivations they occur as oval or spherical bodies, without a distinct capsule, and frequently form a short chain.

Growth takes place most favourably at 37° C. and in the presence of oxygen, although the organism can grow in the absence of this gas. But usually the growth is very feeble on most of the ordinary media, with the exception of blood serum. Blood-agar is a favourable medium—the hæmoglobin of the blood is converted into a chocolate-coloured pigment, which diffuses into the agar. It is in this way that the *rusty* colour of the sputum is produced. The micro-organism also grows in broth and curdles milk.

On artificial media the organism dies quickly, but in dried sputum it may remain alive for long periods. It

is rapidly destroyed by exposure to 52° C., or by a one per cent. solution of carbolic acid.

Mice and rabbits are very susceptible, while pigeons and fowls are immune. No soluble toxin is formed by the micrococcus; and death takes place as the result of septicæmia.

The diplococcus of pneumonia is frequently met with in the saliva of healthy individuals, and it has also been detected in meningitis, ulcerative endocarditis, and in acute abscesses.

CHAPTER XIII.

ANTHRAX.

ANTHRAX was one of the first specific diseases which was proved to be associated with a definite micro-organism, the *bacillus anthracis*. The bacilli were discovered by

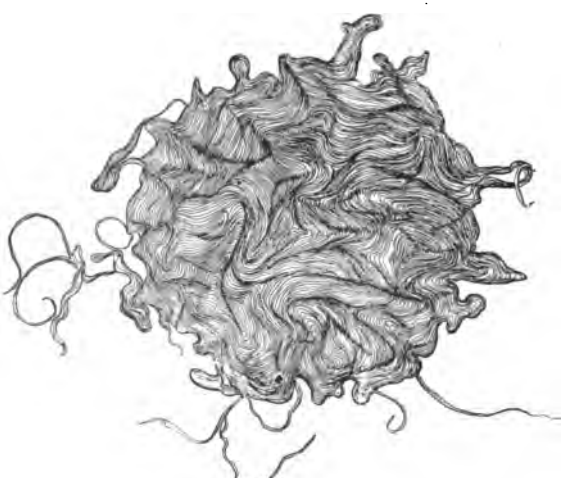


FIG. 8.—Bacillus Anthracis. Plate colonies on glycerine agar-agar. [From Curtis's *Essentials of Practical Bacteriology*.]

Davaine in 1850, but their etiological rôle was only established by the subsequent researches of Pasteur and Koch.

The bacilli (*Frontispiece*, Fig. II.) occur as long rods, measuring from 5 μ to 20 μ in length; in fact, they are the

largest of all known pathogenic bacteria. They usually grow in the form of long threads, the individual bacilli appearing somewhat enlarged at the ends, so that the appearance of a bamboo cane is produced. They are immobile and ærobic, although they may grow anærobically.

Spore formation is an important feature of this organism. Under suitable conditions spores are readily developed in the long filaments, and appear like "peas in their pods". Spore formation never takes place in the body, owing to the lack of oxygen and of a suitable temperature.

On account of their extreme resistance, anthrax spores are frequently used as test objects for determining the value of various germicides. If kept dry and away from light the spores retain their vitality for many years, but they are rapidly killed by exposure to a temperature of 100° C., or to 0·1 per cent. solution of formalin.

In agar plate cultures the bacilli grow into long threads,

FIG. 9.—Bacillus Anthracis. Gelatine stab culture.
[From Curtis's *Essentials of Practical Bacteriology*.]

which give rise to a convoluted tangled mass of growth. The growth in gelatine stab cultures is characteristic: fine filaments grow out laterally from the track of the needle, while liquefaction of the gelatine takes place slowly from the surface. Upon potato a greyish white layer is produced, with abundant spore formation.

As regards *pathogenesis*, cattle and most sheep are highly susceptible, but frogs, dogs, and the Algerian sheep are immune. Man occupies an intermediate position between these extremes. In susceptible animals the disease takes a rapid course, with usually fatal results. The bacilli quickly multiply, fill up the lumen of the capillaries, and produce atypical *septicæmia*. The sanguineous discharges of the infected animal are consequently rich in these bacilli, which finally sporulate on the surface of the ground. The spores are blown about and infect pastures, so that animals grazing on these soils are liable to infection. The spores, unlike the corresponding bacilli, are uninjured by the gastric juice and grow out into rodlets, which enter into the circulation and proceed to multiply.

Infection may also result from breathing air containing anthrax spores, as in the *wool-sorter's disease* of man. The *malignant pustule* is the result of infection through abrasions in the skin, and is consequently most common in hide workers.

The *B. anthracis* can be attenuated by various means, as, for instance, by cultivation at 42° C.—a temperature which is higher than its optimum. Pasteur's *protective vaccine* was first prepared in this manner. There can be no doubt that protective inoculation against anthrax has proved immensely successful in conferring immunity on sheep and cattle.

CHAPTER XIV.

CHOLERA.

THE bacteriology of this disease has only been worked out within a comparatively recent period. For although the infectious nature of cholera had been recognised for a long period, nothing was known of the true nature of the

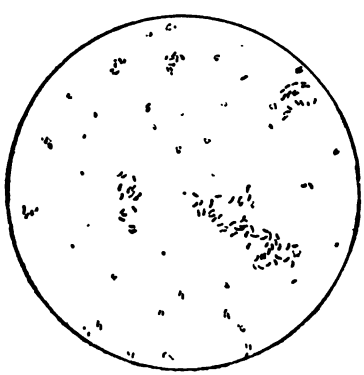


FIG. 10.—Vibrio Choleræ. From a film prepared from cholera "rice-water stool".

[From Curtis's *Essentials of Practical Bacteriology*.]

disease. It was not till 1883 that Koch, by his brilliant researches in Egypt and India, discovered a peculiar organism, the "*comma bacillus*," which is now generally admitted as the *causa causans* of cholera. This organism was constantly met with in all cases of true cholera, and

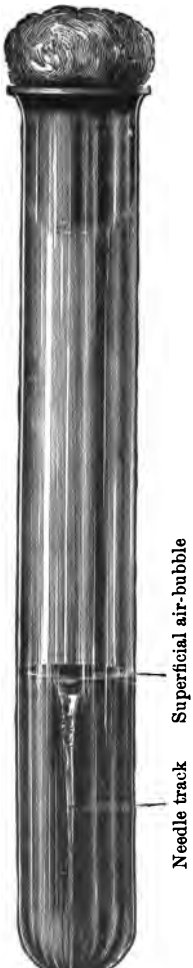


FIG. 11. — Vibrio Choleræ. Stab culture in gelatine (third day).
[From Schenk's *Bacteriology*.]

in no other disease. Where cholera causes most marked changes, *i.e.*, in the lower half of the small intestine, the bacilli were most numerous—above it they diminished more and more. This constant occurrence of the comma bacilli and their limitation to the choleraic process cannot be regarded as an accidental coincidence. On the contrary, the organism and the cholera process must be related to each other as cause and effect.

The comma bacillus, or rather *cholera vibrio* or spirillum (*Frontispiece*, Fig. III.), occurs as a curved rod, singly or in pairs, the latter giving rise to half-circles or S-shaped curves. It is actively motile, and usually possesses a single flagellum. The organism can be readily stained by various aniline dyes, but is decolorised by Gram's method. It does not form spores, although Hueppe claims to have seen small brilliant bodies, which he called "*arthospores*".

Stab cultures in gelatine are characteristic: there is a whitish growth along the needle track with gradual liquefaction, which at first is more marked near the surface, so that a funnel-shaped depression is formed from the resulting evaporation. Liquefaction is comparatively slow,

but after some days it has progressed so far as to destroy the appearance just described.

On agar-agar there is a superficial slimy growth offering no special features. Gelatine plates are very characteristic, and by some are considered to have a distinct diagnostic value. The young colonies show uneven margins, and the surface looks as if it were covered with little fragments of broken glass.

FIG. 12.—Islet of Vibrio Choleræ on a gelatine plate, in process of liquefaction. [From Schenk's *Bacteriology*.]

While the nutrient media on which the cholera spirilla are expected to prosper must be of a marked alkaline reaction, these organisms possess a notable capacity of accommodating themselves to acid media, provided the acid is of a vegetable origin. The surface of boiled potato has often a feeble acid reaction, and yet the vibrio develops on it at the body temperature (37° C.).

Broth and milk are excellent media for the growth of this organism. It also grows readily in peptone water, forming indol and nitrites, which, on the addition of concentrated sulphuric acid, gives rise to a characteristic red colour (*cholera-red* or indol reaction).

Although a resting stage is absent, yet these organisms are more resistant than is generally supposed. In fact, they are so little fastidious in their requirements that, between 17° C. and 40° C., they will grow on almost anything. They, however, thrive best from 35° C. to 37° C., and especially if the medium is faintly alkaline.

In sterilised distilled water the vibrios quickly die out, but the addition of common salt greatly prolongs their existence. When added to sewage they are soon overpowered by the vulgar saprophytic bacteria, but may live from two to four weeks. In ordinary moist soils they

remain alive from one to two months; but in dry soil and peat they die out in a few days.

The vibrios thrive exceedingly well on carbohydrates (*e.g.*, rice), and on such fruits as melons and cucumbers. On the whole, however, the acidity of fruits favours the death of the organism.

They are rapidly killed by desiccation, high temperature (55° C. for ten minutes), sunshine, and the ordinary antiseptics.

Is the Cholera Vibrio the Specific Germ of Cholera ?—(1) It has already been remarked (see p. 60) that an important evidence in favour of the specificity of an organism consists in the experimental production of disease by the use of pure cultures. Now, the lower animals appear to be immune to cholera, and it is, therefore, difficult to reproduce this disease by artificial means. In Bengal, where cholera is endemic, and where domestic animals live in close association with the people, the animals remain remarkably free from cholera.

Koch thought that the acidity of the gastric juice and the intestinal peristalsis were the two conditions which prevented the organism from gaining a foothold in the intestine. To counteract these factors he neutralised the former with sodium carbonate, and paralysed the latter by an injection of opium. On subsequent inoculation with a cholera culture, he was able to produce in guinea-pigs a condition closely akin to cholera. The *post-mortem* appearances more or less resembled those of cholera, but it was noticed that the animals died without having vomited or passed watery evacuations. Guinea-pigs, however, do not vomit; and the absence of diarrhœa is probably due to the extraordinary size of their cæcum, which is capable of retaining considerable quantities of the intestinal contents.

Metschnikoff, struck by the fact that animals very sensitive to subcutaneous or intraperitoneal injections enjoyed an immunity against vibrios introduced by the mouth, thought that this protection was due to the influence of the intestinal flora. Having ascertained the fact that the intestinal canal of newly born rabbits is almost sterile, he fed the young sucklings with cholera cultures, and was thus able to reproduce the classical symptoms of cholera. When the suckling stage is passed, the microbic contents increase with the change of food, and the results are far less satisfactory.

But although the question of experimental inoculation is beset with many difficulties, there are on record many instances of accidental infection in man. In some of these cases a severe attack of cholera followed, and in others death resulted from the infection. On the other hand, several experimenters have swallowed cultures without any injurious effects. But this is in accordance with the fact that not every one exposed to cholera infection is attacked by the disease.

(2) A criticism of a different kind comes from Cunningham of Calcutta, who denies the vibrionic unity of cholera, and contends that the organisms isolated from fresh evacuations are not of one, but of several species, distinct morphologically and biologically.

But these observations do not prove anything more than that cholera organisms are susceptible of great variations. Indeed, the *variability* of the cholera germ is observed not only in different cases of the disease, but also in the same case. Thus, when we examine the intestinal contents of a deceased cholera patient, we find vibrios "in which the external forms, instead of the characteristic comma or spirillum, will vary between a coccus and a single thread; the number and disposition of flagella, the secretion of

acids, and the form of growth on broth will vary; there will be varieties which grow luxuriantly in given media, and others which do not grow there at all; some will be phosphorescent in the dark and others not; some will give the indol reaction, and others will be deprived of this property, and so on" (Haffkine).

(3) The specificity of the cholera spirillum has also been seriously called into question by the fact that vibrios closely resembling it have been detected in the drinking supplies of communities which were not suffering from cholera at the time. Thus, Sanarelli isolated thirty-two such vibrios from the drinking water at Versailles. They were of extreme variability; a few gave indol reaction, others did so after a few days, and the remainder not at all.

The differential diagnosis of these organisms is a matter of considerable difficulty, but Pfeiffer has proposed a test which is satisfactory in most cases. This *test* depends on the fact that the serum of an animal immunised against cholera is protective against this organism, but not against any other species. Thus, if the mixture of an organism and the immunised serum be injected into the peritoneal cavity of a healthy guinea-pig, and a drop of the peritoneal fluid microscopically examined, the organism will be found swollen and disintegrated, if it belongs to the same species as that employed for immunising the animal. As a result it has been found that the majority of Sanarelli's vibrios are the degenerate descendants of true cholera germs, which, perhaps, existed in the course of a former epidemic. What is the precise origin of those organisms which, while giving all the reactions of the cholera vibrio, exist in localities where this disease has never appeared, is a question which cannot be satisfactorily answered.

Of the numerous organisms more or less resembling the cholera spirillum, there are two vibrios, which, on

account of their historic importance, deserve a passing notice. They are the *spirillum of Finkler and Prior* and the *spirillum Metschnikovi*. The former, obtained from the dejecta of patients suffering with cholera nostras, liquefies gelatine much more quickly than the cholera spirillum, and, unlike the cholera germ, grows upon potato at the room temperature. The *spirillum Metschnikovi* obtained from the intestinal contents of chickens also liquefies gelatine more rapidly than the cholera spirillum, and is fatal to pigeons, even in minute doses.

Protective inoculation against cholera by Haffkine's method has yielded very promising results in India. For this purpose Haffkine prepares two vaccines or prophylactic fluids—one, a strengthened vaccine obtained by growing the virus in the peritoneal cavities of a series of guinea-pigs; and the other, a mild vaccine obtained by passing a current of air over the strengthened virus, so as to weaken its pathogenic powers. The ordinary laboratory cultures are usually attenuated, but cannot be employed for inoculation, as they vary widely as regards their virulence. Haffkine's vaccines, on the contrary, are absolutely *fixed* in their value, and herein lies the secret of the success of this treatment.

The weakened vaccine is first inoculated, and is then followed by the strengthened vaccine. A slight reaction occurs after each inoculation, but in no case is there any danger to life.

CHAPTER XV.

TYPHOID.

THE organism of typhoid fever was demonstrated in the spleen by Eberth in 1881, and subsequently isolated and studied by Gaffky. It is, therefore, sometimes called the *Eberth-Gaffky bacillus*. Although it has not hitherto been possible to exactly reproduce the disease in animals, this

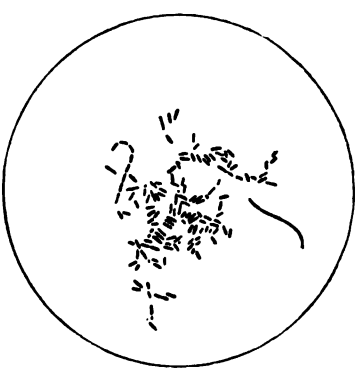


FIG. 13.—Bacillus Typhosus.
[From Curtis's *Essentials of Practical Bacteriology*.]

organism is nevertheless recognised as the veritable germ of typhoid fever.

The *B. typhosus* occurs as a short fat rod, about 2 μ long, and with rounded ends. It is actively motile, and possesses about ten to twenty flagella arranged round the

entire periphery of the organism. It does not form spores, and is stained with aniline dyes, but not by Gram's method.

In gelatine plate cultures small white colonies appear in two or three days, the surface of which are covered with lines and grooves not unlike "the veining of a vine leaf". A uniform turbidity is produced in bouillon, but the growth is most luxuriant in agar and glycerine-agar. On potatoes, having an acid reaction, there is an "invisible growth," which is said to be highly distinctive. Milk is not coagulated, but a slight acidity is produced.

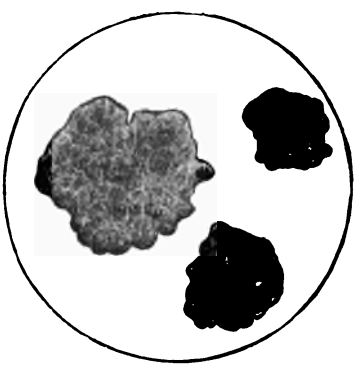


FIG. 14.—Bacillus Typhosus. Gelatine plate culture.
[From Curtis's *Essentials of Practical Bacteriology.*]

The typhoid bacillus is peculiarly resistant, and may linger a long time upon clothing, in stools, and in the soil. It resists the action of gastric juice for several hours, but is rapidly killed by sunlight and various disinfectants.

As animals do not suffer from typhoid fever it is not easy to reproduce this disease by inoculation. However, by increasing the virulence of germs by various means, it is possible to obtain cultures which are truly pathogenic for animals. On the other hand, attenuated cultures

of typhoid bacilli may suffice to produce the disease if the resistance of the animals has been previously lowered by making them inhale sewer gas.

The bacillus occurs constantly in typhoid stools, in the intestinal lesions, as well as in the spleen and liver. It is rarely found in the blood. Locating itself in the intestine it produces poisonous substances, which give rise to fever and the other specific lesions. During the existence of the disease the system *reacts* in a peculiar manner, the exact significance of which is not sufficiently known. The reaction has been studied by Widal, who showed that the serum of typhoid patients causes the bacilli to lose their motility and aggregate into clumps.[1] This is the principle of the *serum diagnosis* of typhoid, which is now extensively employed for the differentiation of this disease.

Protective inoculation against typhoid with the serum of immunised animals has been largely practised by Wright. The subject is still in the experimental stage, but the results hitherto obtained are certainly encouraging.

Relation to Bacillus Coli Communis.—The *B. coli communis*, or the "colon bacillus," is an organism almost identical with the *B. typhosus* in its general appearance, and it becomes important to distinguish the one from the other. The colon bacillus, however, is shorter, has fewer flagella, forms a thick yellow layer on potato, produces gas, and gives the indol reaction.

It is a normal inhabitant of the human intestine, and is usually harmless. But it becomes significantly abundant in cholera and various forms of enteritis. It sometimes

[1] *Agglutination* is also well seen in cholera, plague, and dysentery. The phenomenon may be studied under the microscope or in a test tube, in which case the clumps settle in the fluid in the form of sediment ("*sedimentation*").

escapes from the bowel wall, especially when the latter is injured, and may give rise to peritonitis. It becomes virulent during the presence of typhoid fever, and is the common cause of the suppurative processes occurring in the later stages of this disease.

The close relationship between the typhoid and colon bacilli makes it highly probable that both organisms are derived from a common ancestor. As a matter of fact,

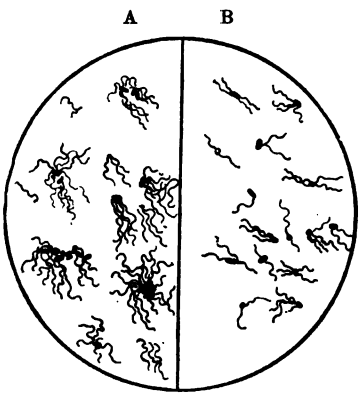


FIG. 15.—A, Bacillus Typhosus. B, Bacillus Coli Communis. [From Curtis's *Essentials of Practical Bacteriology.*]

they may be said to form one great group, out of which the typhoid bacilli have arisen by natural selection. How far the *B. typhosus* is helped by colon bacilli in the production of enteric fever is a question on which there is a considerable diversity of opinion. But it may be affirmed that the *B. coli communis* does bring about some of the symptoms of this disease, as it is met with in practice.

CHAPTER XVI.

TUBERCULOSIS.

TUBERCULOSIS was regarded as an infectious disease even by the older physicians; but it was Koch who, in 1882, discovered the specific organism and established the fact that without the *B. tuberculosis* there could be no tubercle.

Tubercle bacilli (*Frontispiece*, Fig. IV.) are fine rods having a length of about half the diameter of a red-blood corpuscle, and occur singly or in pairs. They take up the ordinary stains very slowly, but after being stained they are not decolorised even by mineral acids ("*acid-fast*"). When stained, they are frequently beaded, the unstained portions probably representing the vacuoles rather than spores. In old cultures the bacilli tend to be filamentous, and may also show true branching, or they may be swollen at one extremity. These appearances have been variously interpreted; Metschnikoff holding that "the bacillus, as ordinarily met with, is not the end-stage, but only a stage in the developmental cycle of a filamentous fungus". Other observers, on the contrary, regard these aberrant forms as mere degenerative changes which normally occur in the life-history of an organism.

Being a typical parasite, the organism grows on blood serum, but not on ordinary media. These, however, can be made available for its culture by the addition of glycerine. It will then grow on bouillon, agar, potato, and even on such substances as carrot and macaroni.

The growth on glycerine-agar is highly distinctive: small dry scales appear at the end of two weeks, which finally coalesce and form a white wrinkled membrane. The growth on blood serum is similar, but proceeds more slowly.

Although spore formation has not been demonstrated, tubercle bacilli are fairly resistant outside the body. They remain alive in dried sputum for two to three months, and resist the action of gastric juice and putrefactive bacteria. On the other hand, they are easily destroyed by sunlight and various germicides. Like other pathogenic germs which exist in milk, they are killed by the heat of "*pasteurisation*" (70° C. for thirty minutes).

Pathogenesis.— Tuberculosis can be readily produced in various animals by subcutaneous or intraperitoneal injections, or by feeding the animals with cultures, or by making them inhale the dried bacilli. Subcutaneous injection, however, is the mode commonly employed for diag-

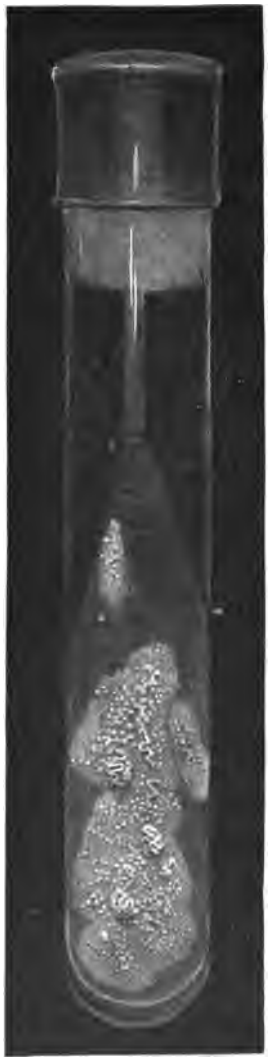


FIG. 16.—Bacillus Tuberculosis. Glycerine-agar culture, several months old.
[From Curtis's *Essentials of Practical Bacteriology.*]

nostic purposes in the case of sputum or urine suspected to be tuberculous. Thus, when tuberculous sputum is introduced beneath the skin of a guinea-pig there occurs a local tuberculosis at the seat of inoculation, followed by infiltration of the lymphatic glands, which progresses in a definite order. The animal becomes emaciated, and finally succumbs to general tuberculosis in the course of six to twelve weeks.

In the case of intraperitoneal injections there are no local manifestations, and death is more rapid.

Besides guinea-pigs, rabbits, birds and most domestic animals can be infected with tuberculosis, although man, the monkey and cattle are most subject to the disease.

In *man* the disease mostly results from inhalation of the dried tubercular *sputum*. Such sputum usually contains living bacilli for several months, and is very infective. It has been demonstrated that in the dust of consumption hospitals bacilli are present in sufficient numbers to induce tuberculosis in guinea-pigs. According to Flügge, an important source of infection is to be found in the fine atoms of moisture discharged by consumptive patients during coughing and sneezing. Their existence can be readily demonstrated by holding a mirror before the face; but they do not generally pass further than about twenty inches from the patient.

Another mode of infection is from the ingestion of tuberculous *milk* or *meat*. But Koch has recently declared that human tuberculosis is different from the bovine, and that man is so rarely infected from the latter that it is unnecessary to take any measures against it. These views have not yet been confirmed—they are, indeed, open to grave doubt—and in the meanwhile it will be best not to relax our precautions in this matter.

Toxins.—It has been found that intravenous injections

of dead tubercle bacilli in rabbits give rise to pulmonary tuberculosis, which closely resembles the lesion produced by the living organisms. It thus appears that the toxins of *B. tuberculosis* are chiefly *intracellular*, that is to say, are contained in the bacterial cell, and do not diffuse into the surrounding medium.

Koch's original "*tuberculin*," which is the filtered glycerine-broth culture of the organism, probably contains certain toxins derived from the bacilli. It has no effect on healthy persons; but, if tuberculosis or lupus is present, fever and local necrosis round tubercular deposits follow its injection. The reaction is not specific, for it is also produced when the person is suffering from syphilis, and when milk and ricin are substituted for tuberculin. The tuberculin reaction, however, is extremely useful in detecting latent tuberculosis in cattle, and, for this purpose, is largely employed on the Continent.

Koch has recently introduced other tuberculins, which are a considerable improvement on his original preparation. The most important of these is the "*tuberculin R.*" (T. R.), which is prepared by repeatedly crushing the dried bacilli, so as to extract their intracellular toxins. This tuberculin has been used in early phthisis and lupus, but without any satisfactory results. In the laboratory, however, it has proved successful in conferring immunity on guinea-pigs experimentally inoculated with the tubercle bacillus.

Relation of the Human Tubercle Bacillus to Allied Organisms.

(1) *Avian Tubercle Bacillus.*—The organism of avian tuberculosis is apparently similar to the human tubercle bacillus, but differs from it in some essential particulars.

In addition to certain morphological and biological differences, an important point with regard to this organism is that it is pathogenic for fowls and harmless for man.

The two types of bacilli, however, do not form distinct species, but are only varieties modified by growth in the tissues of different hosts. This is now established by the experiments of Nocard, who, by growing human tubercle bacilli in the peritoneal cavities of a series of fowls, was able to convert them into the avian type.

(2) *Bovine Tubercle Bacillus.*—As has already been remarked, Koch recently stated that the bacilli of human and bovine tuberculosis are essentially different from each other. He, therefore, believes that human tuberculosis differs from the bovine, and cannot be transmitted to cattle. This is nowadays accepted by most authorities.

But the converse of the proposition, *i.e.*, the insusceptibility of man to bovine disease, has not been definitely proved. On the contrary, cases have frequently been recorded in which man has been accidentally inoculated with the bovine tubercle. The question, however, must be regarded as *sub judice*, although most workers are disinclined to accept Koch's observations.

(3) Recently, certain organisms have been obtained from various species of grass, milk and manure, which bear a remarkable resemblance to the tubercle bacillus. They present the same staining reactions (*acid-fast*), and on inoculation into guinea-pigs give rise to tubercle-like nodules. But unlike tubercle bacilli they grow quickly on artificial media, showing that these organisms are not parasites.

CHAPTER XVII.

LEPROSY.

THE essential cause of leprosy is an organism, the *bacillus lepræ*, discovered by Hansen in 1879. It closely resembles the organism of tuberculosis in form and staining reactions, although differing from it in some essential particulars. Both bacilli are "acid-fast," and their pathological lesions are more or less alike. But the leprosy bacilli are somewhat thinner, stain more easily, and are usually arranged in bundles. They lie chiefly in the lepra cells, but may also be seen in the lymph spaces (*Frontispiece*, Fig. V.). They are never found free in the blood, a fact which may explain the chronic nature of the disease and the difficulty of its communication to healthy subjects.

The bacilli are present in the discharges from ulcers, and probably also in the nasal and salivary secretions. Nothing is known of their distribution outside the body. They have not been detected in the soils on which the lepers reside, or in the water in which they bathe. Examinations of fish have also given negative results. Flies feeding on leprous ulcers fail to show the bacilli.

The leprosy bacillus has not been cultivated outside the body, and all attempts to reproduce the disease in animals have failed. Some experiments have been made by inoculating with portions of leprous tissue the vascular combs of fowls and the anterior chambers of rabbits' eyes, but

beyond a local infiltration no other results followed. In no case a definite generalised leprosy has, thus far, been produced.

But this only shows that leprosy is essentially a human disease and does not occur in lower animals. The failure to cultivate the specific bacillus outside the body is probably due to the fact that the organism, being a strict parasite, requires a more specialised pabulum than that afforded by our artificial media. Although the exact proof of an etiological relationship is wanting, the universal presence of the bacilli in the tissues, its absence in healthy persons, and its intimate association with the pathological lesions, warrant the assumption that it is the essential cause of leprosy.

CHAPTER XVIII.

ACTINOMYCOSIS.

This disease, which occurs commonly in cattle, and not unfrequently in man, is caused by a specific organism—the *streptothrix actinomyces*, or the "ray fungus". This is not a true bacterium, but a streptothrix, and is characterised by a filamentous growth which gives rise to a felted mass of structure. The individual filaments are

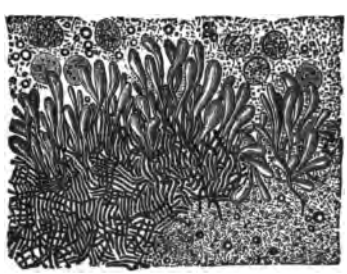


Fig. 17.—An Actinomyces Granule. Unstained preparation. [From Schenk's *Bacteriology*.

frequently branched, and at the free ends may show pear-shaped swellings ("*club formation*"). Occasionally the threads segment into coccus-like bodies, from which new individuals develop.

The fungus grows on barley and other cereals, and by these means infects both men and animals. It gains

access to the tissues chiefly through abrasions of the mucous membrane of the mouth, but there is no tissue or organ which may not be attacked by the fungus. In cattle the disease is usually localised, and is of the formative type. A favourite seat of lesion is the tongue, which becomes extremely hard and indurated ("wooden tongue"), but the palate and skin may also be attacked.

In man the disease frequently affects the lower jaw, but the inflammatory tissue, instead of forming nodules, usually breaks down into pus and gives rise to abscesses.

Actinomyces grows in tissues in round yellowish masses, which are just visible to the naked eye, and appear like grains of iodoform. If one of these bodies be flattened out and examined under the microscope it is seen to be made up of a central network of branching filaments enclosing coccus-like bodies, and forming at the periphery a fringe of club-shaped processes. The clubs are formed by the swelling of the sheath of the fungus, as the result of degenerative changes. They are frequently calcified, and are more often met with in the bovine than in the human variety of the disease.

The fungus readily grows on artificial media. On agar the colonies are discrete, of a yellow beeswax colour, and usually very hard. On potato the growth is abundant and of a yellowish or brownish colour. Bread paste is also a favourable medium.

The results of inoculation are frequently unsatisfactory. The fungus has not been found outside the body, and nothing is known of its life-history.

Madura Disease.—This is a chronic local affection, frequently affecting the foot, which is enlarged and shows numerous fistulous openings discharging a thin sanious liquid containing peculiar cellular bodies. These are sometimes of a yellowish and sometimes of a black

colour, and on this account the white and black varieties of the disease are recognised.

When examined under the microscope the little bodies are seen to be fungoid in nature. It appears that the two varieties are not caused by the same organism, but by distinct species of streptothrix, both of which, however, are closely related to the actinomyces.

As in actinomycosis, the disease is communicated through the skin when the latter is injured by thorns, prickly plants, etc., infected with the fungus.

CHAPTER XIX.

DIPHTHERIA.

If a serum tube be inoculated with a swabbing from the throat of a patient suffering from diphtheria, and then incubated at 37° C., a growth takes place in the course of a few hours. On staining with methylene blue the specific organisms, or *Klebs-Löffler bacilli*, are readily

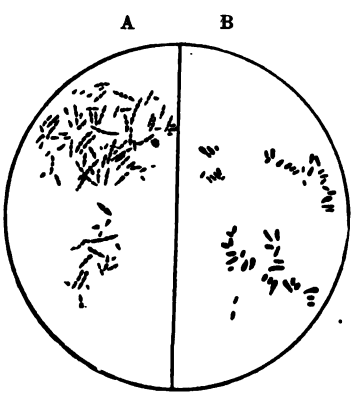


Fig. 18.—Bacillus Diphtheriæ. A, long variety; B, short form. [From Curtis's *Essentials of Practical Bacteriology*.]

seen under the microscope. Their presence is diagnostic of the disease, but their absence does not prove the contrary.

The bacilli occur as straight or slightly curved non-motile rods, which are frequently "clubbed" at one or

both extremities, and show a "beaded" appearance on staining. In film preparations the organisms are usually arranged in characteristic groups (Chinese letter formation). The individual bacilli may be long or short; and various involution forms may not unfrequently be seen in the same culture. These irregular forms are very commonly observed, and may be said to be characteristic of this organism.

The diphtheria bacillus is ærobic and does not form spores. It grows best on blood serum, although it can also grow on gelatine, agar, broth, and other media. On blood serum small circular white colonies develop within a few hours. Similar colonies also develop on agar-agar, but the growth takes place rather less rapidly. In broth it produces a turbidity which, however, soon settles to the bottom. Milk is a favourable medium and may serve as a carrier of infection.

The organism is rapidly killed under the influence of light, heat, and various germi-

FIG. 19. — Bacillus Diphtheriæ. Pure culture on serum, about 36 hours old.
[From Curtis's *Essentials of Practical Bacteriology*.]

cides, but in a dried diphtheritic membrane it may retain its vitality for weeks or months.

Diphtheria is essentially a *local* disease, the specific bacilli being present only at the seat of inoculation, and very rarely in other tissues. There they manufacture the toxins which, being absorbed into the blood, produce the clinical symptoms of the disease. The paralytic symptoms which occur in the course of the disease have been experimentally produced in animals by the use of separated toxins. But it appears that the bacilli, even in pure cultures, cannot induce the formation of a false membrane unless the mucous membrane is directly injured. It is, probably, in this manner that the *pyogenic cocci*, so frequently met with in the false membrane, injure the tissues and pave the way for diphtheritic infection. The cocci grow side by side with the specific organisms, and may thus increase the virulence of the latter, and bring about some of the complications of this disease.

The antitoxin or *serum treatment* has proved eminently successful in the cure of diphtheria. But the treatment, to be successful, must be commenced at the earliest opportunity in order to prevent the combination of the toxin with the tissue elements, for which it has a selective affinity. As the interval between infection and the introduction of antitoxin increases, so the value of the latter rapidly diminishes, till finally it can no longer prevent death. The antitoxin, however, only neutralises the toxin of diphtheria, and has no action against the septic symptoms produced by the associated streptococci. For this reason it has been recommended to administer antistreptococcus serum along with the diphtheria antitoxin.

Organisms are occasionally met with in healthy throats which are indistinguishable from the true diphtheria

bacilli in their morphological and cultural characters, but differ from them in the fact that they are non-virulent. It has been suggested that these *pseudo-diphtheria bacilli* form a distinct species, but the balance of opinion inclines to the view that they are merely the attenuated forms of the Klebs-Löffler bacillus.

CHAPTER XX.

GLANDERS.

GLANDERS occurs chiefly among horses and asses, but may be communicated to persons associated with the infected animals, such as stablemen and grooms. The infective material is present in the discharges from the nostrils and in the pus from the nodules.

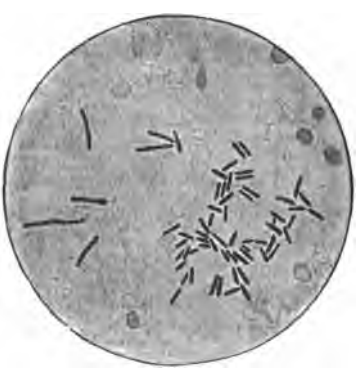


FIG. 20.—Glanders Bacilli in a section of lung.
[From Curtis's *Essentials of Practical Bacteriology*.]

The exciting cause is a bacillus somewhat shorter and thicker than the tubercle bacillus. The *bacillus mallei* stains readily with the ordinary dyes and frequently shows a "beaded" appearance on staining. It is decolorised by Gram's method of staining. It is a strict parasite and does not form spores.

The *B. mallei* does not grow in infusions of hay or horse manure, but can be cultivated in glycerine-agar and blood serum. The growth on potato is very characteristic. On this medium, at incubation temperature, the growth readily appears as a honey-like layer, which later changes to a brownish or chocolate colour.

Glanders can be easily communicated to laboratory animals, and accidental infection in man has frequently been recorded. It is said that lions and tigers in menageries have contracted the disease from being fed on the flesh of infected animals.

Drying, high temperature, and antiseptics quickly kill the glanders bacillus. The organism has never been found outside the body.

A toxic substance (*mallein*) has been obtained from cultures of the bacillus. This, when injected into infected animals, acts like tuberculin when used for the detection of tuberculosis.

CHAPTER XXI.

INFLUENZA.

THE *bacillus of influenza* was discovered by Pfeiffer, in 1892, in the purulent bronchial secretions of infected patients. It is the shortest of all known bacilli, and on account of its minute size may be mistaken for a coccus.

FIG. 21.—Influenza Bacillus. From a film prepared from influenza sputum.
[From Curtis's *Essentials of Practical Bacteriology*.]

The bacilli are usually arranged in chains. They are ærobic and do not form spores.

Artificial cultures are made with difficulty. The best medium is blood-agar, which, after inoculation with the sputum, shows characteristic colonies at the end of twenty-four hours. These, when observed with a lens, are seen

to consist of minute transparent dots, like drops of dew, which always remain separate from each other. "This feature is so characteristic that the influenza bacilli can be thereby with certainty distinguished from other bacteria" (Kitasato).

The influenza bacillus is quickly destroyed by antiseptics and drying, although in the moist sputum it may remain alive for two to three weeks.

Although the disease cannot be reproduced in animals, there can be little doubt that Pfeiffer's bacillus is the essential cause of influenza. The bacilli are mainly *localised* in the respiratory tract, where they produce their toxic metabolic products which, as in the case of *B. diphtheriæ*, have a selective affinity for the nervous tissue.

CHAPTER XXII.

PLAGUE.

THE bacteriology of this disease has been worked out only within the last few years. The specific organism was discovered in 1894 by Kitasato and Yersin, being constantly met with in the enlarged glands, blood, and internal organs. In the pneumonic form of the disease it is present in the sputum.

In form the *bacillus pestis* (*Frontispiece*, Fig. VI.) is a short oval rod, rounded at both extremities. In specimens from blood it is usually arranged in pairs, giving rise to the appearance of diplococci. But in artificial cultivations it frequently forms chains of varied lengths.

It is readily stained by aniline dyes, but the extremities take on a deeper hue than the intervening portion ("polar staining"). It does not form spores, and, according to Kitasato, is actively motile.

In cultivations the organism is especially prone to undergo degenerative changes and give rise to various involution forms. It, however, grows well on ordinary media. In broth it gives rise to a granular deposit on the sides and bottom of the tube. On agar-agar (and better still, on glycerine-agar) it forms an abundant cream-coloured growth which, when viewed from behind, presents a dull, silvery appearance. Haffkine has found that if broth cultures, containing a little *ghi* or cocoanut oil, are kept absolutely at rest, the organisms grow in a most charac-

teristic fashion. They attach themselves to the floating drops of butter forming little islands of growth, from which they grow down in the medium in the form of long threads producing the appearance of *stalactites*. If the culture be shaken the stalactites fall to the bottom, but they are reformed on subsequent inoculation.

The disease can be communicated to most laboratory animals, but *rats* and *mice* are particularly susceptible. As is well known, the epidemic is frequently preceded

FIG. 22.—Bacillus Pestis. Culture in butter broth showing stalactites.

and accompanied by the sudden death of many of these animals. It is stated that the nomadic tribes on the northern slopes of the Himalayas, even to-day, when they observe an extraordinary number of dead rats, are so certain of an approaching epidemic that they immediately vacate their quarters and shift elsewhere.

Monkeys can be easily inoculated with the plague—a single puncture with a needle dipped in a culture of *B. pestis* being sufficient. The importance of this observation in relation to infection in man is self-evident.

Plague bacilli have been detected in the dead bodies of fleas, but the majority of observers agree that suctorial insects play no part in the transmission of the disease to man.

Birds and bovines are immune. It is probable that vultures feeding on the corpses of the plague-stricken suffer no ill effects, but they may spread the disease by means of their excreta.

Like other spore free organisms the bacilli are readily killed by desiccation, heat, and ordinary antiseptics. A solution of sulphuric acid (1 in 250) is especially recommended by Hankin as a cheap and efficient germicide. The bacilli are also destroyed by direct sunlight in three or four hours. But it is doubtful if any of these measures can be absolutely relied upon during an epidemic.

On account of their slight resistance and absence of spores infection usually occurs by close contact. The *skin* is the most frequent path, a slight wound or abrasion being sufficient for inoculation. In a few instances the disease may be produced by the entrance of bacilli into the respiratory passages, the organisms being transmitted not in the form of dust, but in small drops of moisture diffused by plague patients (*Flügge's drop infection*, see p. 86).

It is improbable that in human subjects infection ever takes place by means of ingesta, although experiments in animals have yielded positive results.

In regard to the spread of the disease from infected cases, it may be stated that the bubonic variety is relatively unimportant, for in this case the bacilli are locked up in the buboes, and when these break down into pus most of the bacilli likewise perish. In septicæmic cases, on the other hand, the bacilli escape from the circulation and pass out of the body by means of sputum, urine,

stools, etc. It is for this reason that the pneumonic form of plague so frequently leads to the spread of infection.

Prophylactic Measures.—In addition to isolation, disinfection, and other sanitary measures, an important point is the destruction of rats and mice. Whether the infection is carried to man directly through external wounds, or indirectly by means of fleas infesting rats, is not clear. But whatever be the precise mode of human infection there can be little doubt as to the immense danger of harbouring infected rats in a locality. Rats can be exterminated by the usual methods, or by soaking bread with "*Danysz rat virus*," which is the culture of a virulent variety of an organism allied to the *B. coli communis*.

As regards specific prophylaxis, *Haffkine's vaccine* may be recommended. For, although absolute protection is not afforded by the inoculation, both the incidence and case mortality are considerably less in the inoculated than in the uninoculated. The duration of protection is said to be about six months.

The vaccine is essentially a sterilised culture of the *B. pestis* in broth (prepared from goat's flesh), the microbe being grown in it for four to six weeks. The flasks are shaken every few days so as to break the stalactite growths and induce fresh crops. The culture is then sterilised, and a small quantity of antiseptic added.

Haffkine's prophylactic fluid thus contains not only the dead organisms, but also the broth in which they have been cultivated. In other words, it is a mixture of intra- and extracellular toxins, although it is doubtful if the organism forms any soluble poisons. The fluid, when introduced into the body, is assumed to produce

antagonistic substances which neutralise the infective agent should it attack the individual.

Various curative sera (Yersin's, Lustig's) have been tried, but without much success. The antitoxic treatment of plague; therefore, cannot be regarded as a success.

CHAPTER XXIII.

TETANUS.

THE *bacillus tetani*, which was first isolated by Kitasato, is a slender organism usually growing in the form of long threads. The threads are motile, but after growing for some time at 37° C. the motility ceases and the spores are formed. These are rounded in form and are situated

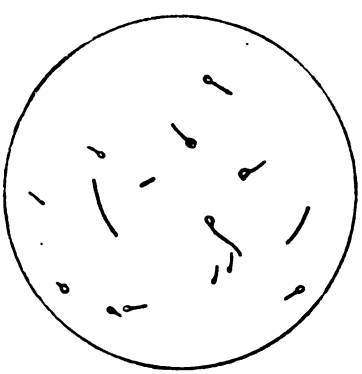


FIG. 23.—Tetanus Bacilli and Spores.
[From Curtis's *Essentials of Practical Bacteriology.*]

at one end of the bacillus, giving it the appearance of a "drumstick".

This organism differs from those we have studied previously in the fact that it only grows in the absence of oxygen (*obligatory anærobe*). It may be cultivated in

an atmosphere of nitrogen (obtained by absorbing oxygen by alkaline permanganates), hydrogen, or carbon dioxide gas. The growth takes place in ordinary media, but the addition of grape sugar gives better results.

In glucose-gelatine stab cultures the growth takes place along the track of the needle, but at a considerable distance below the surface, in the form of a radiating outgrowth. The medium is liquefied, and some gas may be produced. In gelatine plates the colonies are very characteristic: there is an opaque central portion which is surrounded by a series of radiating filaments. The growth also occurs in bouillon and gives off a peculiar fœtid odour.

The *spores* of the tetanus bacillus are extremely resistant. They withstand desiccation for months, and are uninjured after exposure to 80° C. for one hour, but are rapidly killed at 100° C. They resist for many hours the action of 5 per cent. carbolic acid, or 1 in 1,000 of corrosive sublimate; but the addition of a little hydrochloric acid to these substances readily brings about their destruction.

The bacillus leads a saprophytic existence in garden soil and in dung heaps, whence it finds its way into the human organism. But the bacillus alone is unable to give rise to infection—it must be introduced with the pyogenic cocci (*mixed infection*), or there must be some injury to the tissues before it is enabled to gain a foothold and produce disease.

The organism is not found in the blood and tissues, but remains *localised* at the seat of inoculation. In this situation it manufactures the toxins which enter into direct combination with the central nervous system, and thus give rise to the characteristic symptoms of the disease. Wassermann, by mixing tetanus toxin with an emulsion of spinal cord, found, on inoculation into guinea-

pigs, that the mixture was no longer toxic. This shows that there exist in the central nervous system certain molecules with a combining affinity for the tetanus toxin. The experiment affords an interesting confirmation of Ehrlich's hypothesis of immunity (see p. 124).

The results of *serum treatment* are not so satisfactory as in diphtheria. This is due to the fact that, unlike the latter disease, tetanus cannot be diagnosed sufficiently early to allow the antitoxin to have its full scope. An apparently trivial wound is often neglected, and the patient does not come for treatment until the disease is far advanced.

Attempts have recently been made to obtain better results by the *intracerebral* injection of antitoxin, which is also injected hypodermically at the same time. "The intracerebral injection immunises the higher nerve centres before the toxin has been fixed there. The antitoxin given hypodermically renders the blood antitoxic, and the toxin, as it becomes absorbed from the source of supply—wound, bruise, or any other source—is neutralised as soon as it enters the blood" (Semple).

A few successful cases have been recorded as the result of this treatment.

CHAPTER XXIV.

MALARIA.

THE parasite which is now universally acknowledged as the cause of malarial fevers is not a bacterium, but a protozoon called plasmodium or *hæmamœba malariæ*. This organism, which is quite unlike any we have considered before, was discovered by Laveran in 1880. He noticed that it developed in, and at the expense of, the red-blood corpuscle, which was finally reduced to a mere shell, the parasite appropriating the hæmoglobin and blooming into a "rosette". Subsequently, Golgi discovered that these marguerite-like bodies represent the reproductive stage of the parasites, which, moreover, were of various kinds. He also demonstrated the remarkable fact that the malarial paroxysm always coincided with the sporulation of a group of parasites. This cleared up the mystery of the periodicity of this disease; but an important question still remained to be answered. How did these organisms enter the human body? Manson, in 1896, suggested that the mosquito probably subserved the malarial parasite in the same way as it did in the case of *filaria nocturna*. The truth of this hypothesis was subsequently confirmed by Ross's experiments, which showed that a particular species of mosquito served as the carrier of this disease.

The malarial parasite is a unicellular organism consisting of protoplasm, a nucleus, and a nucleolus. It possesses

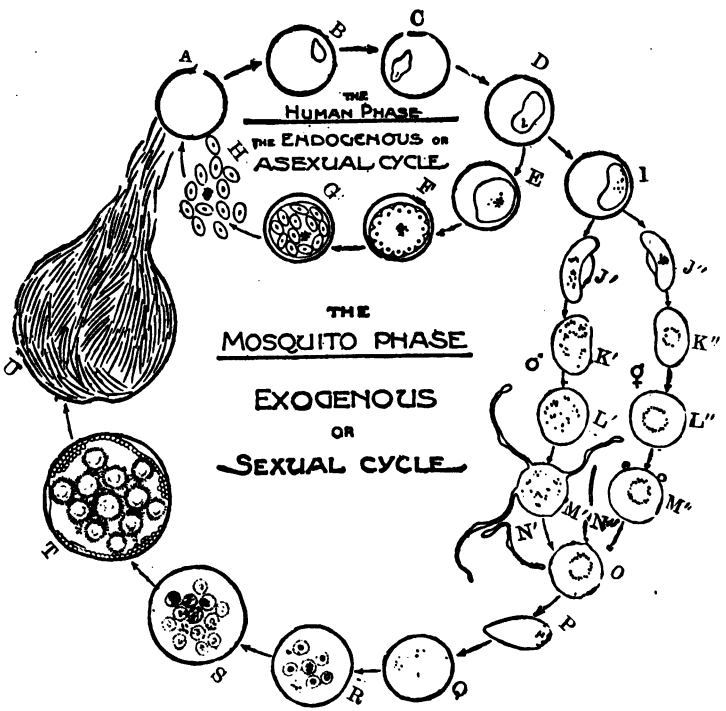


FIG. 24.—Schema showing the Human and Mosquito Cycles of the Malaria Parasite.

A, normal red cell.
B, C, D, E, red cells containing malaria parasites.
F, G, H, sporocytes.
J′, K′, L′, M′, male gametes.
J″, K″, L″, M″, O, female gametes.
N′, N″, microgametes.
P, travelling vermicule.
Q, R, S, T, young zygotes.
U, mature zygote containing blasts.

two cycles of development, one in man and the other in the mosquito. In the human host it propagates by the asexual mode of reproduction; but the species is perpetuated by its passage through the mosquito, in which the reproduction is a sexual one. As has just been stated, it is by means of this insect that the parasite passes from man to man.

Developmental Cycle in Man.—In its earliest stages the malarial organism is seen as a hyaline amœboid body in the substance of the red corpuscle. The organism increases in size and converts the hæmoglobin into melanin, which it appropriates to itself.

When the full maturity is obtained it becomes either a *sporocyte* (sporulation form), or a *gametocyte* (sexual form). In the former case it divides into a number of segments or spores, which, by the rupture of the corpuscular host, are set free to attack fresh corpuscles, and there to undergo a similar cycle of changes. The melanin, which is also discharged into the blood plasma along with the spores, is ingested by the leucocytes. The sporulation of a group of parasites is accompanied by a paroxysm, which is probably due to certain toxins liberated at the same time.

The Mosquito Phase.—The *gametocytes*, on the other hand, show no segmentation, but circulate unchanged in the blood. They represent the sexual forms of the organisms, and become active when transferred to their definitive host, the mosquito. When, therefore, they are imbibed by a mosquito biting a malarial subject, they burst from their corpuscular host, and lie free in the stomach cavity of the mosquito. The male gametocyte now emits a number of active motile filaments called *microgametes*. Some of these spermatozoon-like bodies become detached, and entering the female sexual elements, bring about their fecundation. The resultant fertilised body becomes

elongated and pointed at one extremity, and also exhibits motility. It is called the *travelling vermicule* or *zygote*. It works its way through the mass of blood and the stomach wall, finally arriving at the outer surface of the organ. Here it becomes encysted and develops a large number of delicate spindle-shaped cells called *blasts*. The capsule then ruptures, and the blasts are discharged into the body cavity of the insect. These are finally carried by the circulation to the salivary gland of their host, whence they pass through the salivary duct into the proboscis of the insect. When the mosquito again bites an individual the salivary secretion passes into the wound, and so the blasts enter into the circulating blood of the human host and give rise to the amœboid organisms. These enter the red corpuscles and pursue the developmental cycle described above.

The mosquito, or gnat, belongs to the genus *culicidæ*, of which numerous species are extant. From a medical point of view, only two varieties are important, the *culex* and the *anopheles*. In the early stages of existence, *viz.*, as ovum, larva, and pupa, the mosquito is aquatic; but the mature insect or imago is aerial.

The *culex* or the common house mosquito is found almost everywhere. Its ova are usually arranged in boat-shaped masses, and the larvæ usually live in pots, tubs, and other artificial collections of water. In the adult insect the wings are unspotted, the palpi short, and, when at rest, it is usually found parallel to the wall.

The *anopheles*, on the other hand, is rarer, and serves as the definitive host of the malarial parasite. It is not domestic in habits like culex, but breeds in puddles, rice-fields, or other similar collections of water. The ova are generally in star-shaped or irregular masses, but the

grouping is never boat-shaped. The full-grown anopheles is more graceful than culex, its palpi are long and the wings are dappled with dark spots. It is nocturnal in its habits, and only visits habitations in the evening. Both sexes feed on fruit; but the female alone sucks blood from men and other animals.

Varieties of the Malarial Parasite.—Three distinct species of the malarial parasite are recognised, each of which gives rise to a specific type of infection.

FIG. 25.—Anopheles Claviger.
[From Celli's *Malaria.*]

1. *The Parasite of Tertian Fever.*—The tertian parasite is fully matured in about forty-eight hours, and the sporulation of a group of organisms occurs at the same time, giving rise to the characteristic paroxysms every third day. During its development certain forms are evolved, which are highly characteristic.

If a specimen of blood from a case of tertian fever be examined at or before the period of rigor, it will be observed that many of the red corpuscles contain small hyaline forms actively changing their shape and position

in the interior of the cells. These bodies represent the earliest stages of the parasite, and are derived from the segmentation of the preceding group of the organisms. As the parasite increases in size, pseudopodia are protruded and retracted, so that various characteristic forms arise. Usually a single parasite is contained in a cell, although occasionally two or more are seen.

The next stage in the development of the organism is the collection of dark-brown pigment, which it derives from the hæmoglobin of its host. The pigment granules are more marked at the periphery than at the centre, and their active "brownian" movement constitutes an important feature of the organism.

As the parasite matures, it becomes larger in size, more pigmented and less amœboid, until the latter characteristic is altogether lost. The pigment now becomes coarser, and, instead of being distributed eccentrically, is now scattered through the protoplasm of the parasite. At the same time the infected blood cells are decolorised, so that they appear as pale rims encircling the mature parasite.

The nutritive supply within the blood corpuscle being exhausted, the parasite now proceeds to sporulate. The pigment collects into a central mass, and fine striations are seen to extend from the periphery to the centre, so that the parasite assumes the form of a "rosette". This is the appearance which is observed on examining the blood just before the next paroxysm. The protoplasm of the parasite finally breaks up into twelve to thirty segments, each of which contains a portion of nuclear matter, and is called a spore. The envelope of the red corpuscle ultimately ruptures, and the spores are set free in the blood plasma, in which situation they are seen forming an irregular group round the pigmented mass.

Sooner or later they re-enter red corpuscles, and initiate a similar cycle of forty-eight hours' duration. Most of the liberated pigment is taken up by the phagocytes or deposited in various organs.

Besides these forms, which constitute the asexual cycle of the parasite, certain large pigmented spheres are seen, especially in the apyrexial period. They are the *gametocytes* or gametes, and are derived from the parasite losing its corpuscular envelope prior to sporulation, which, however, does not take place. Some of these gametes emit three or four long delicate flagella, which actively lash about in the fluid, and finally break away from the parent cell and are lost from view.

Certain other spheres may also be seen which are larger and do not flagellate. Their pigment is less active and usually arranged in the form of a ring. They are the female elements, whereas the flagella of the flagellating body represent the male sexual organs, which bring about the fertilisation of the former in the manner already described.

2. *The Parasite of Quartan Fever* completes its cycle of development in seventy-two hours, and the fever is therefore more benign than the tertian. The developmental changes in the parasite are essentially similar to those described above, but a few morphological differences may be observed. Thus, the parasites are smaller, their movements less active, and the pigment granules coarser. The sporocyte or rosette body is made up of from six to twelve spores, and the red corpuscles do not become pale.

3. *The Parasite of Malignant or Æstivo-Autumnal Fever.*—In this type of fever the marked periodicity of the above two varieties is wanting, as larger numbers of this organism exist in the blood in different stages of development. Segmentation thus takes place at different

intervals and gives rise to that *irregularity* which is so characteristic of this disease.

The developmental cycle of this parasite, moreover, is difficult to follow, as the latter portion of its life-history is carried on in the internal organs. However, certain well-marked differences can be made out. The young parasites are very small but very active, and the *ring forms* are common. The red corpuscle usually contains two or more parasites and assumes a characteristic "brassy" tint.

An important feature is the presence of "*crescentic bodies*". They appear in the peripheral circulation after the fever has lasted for some time, and are peculiarly resistant to the influence of quinine. The crescents are nothing else than the gametes, male and female. When examined under the microscope the crescents change into spherical bodies, some of which proceed to flagellate and the others do not. The manner of fecundation is essentially the same as in the tertian and quartan types of fever.

Relations to Disease.—The *hæmamœba malariæ* has not been cultivated outside the body, but its causal relation to the disease may be taken as fully established. Thus, the parasite is always present in the blood of malarial patients and is met with in no other condition. Its cycle of development corresponds with the clinical course of the disease, and the destruction of the red corpuscles fully explains the subsequent anæmia. Quinine, which is a well-known amœbic poison, kills the parasite and cures the malarial fevers. When a healthy person is inoculated with a minute quantity of malarial blood, not only the infection but also the specific type of the disease is reproduced. A malarial person can mix freely with healthy persons without infecting them with the disease, provided there are no anopheles about to convey the parasite.

Although the parasite is undoubtedly transmitted from man to man by the agency of mosquitoes, it is not certain if this is the only mode of propagation of the disease. It may be that the malarial organism has an extra-corporeal existence other than that in the mosquito. It is difficult to speak dogmatically on this subject, but certain considerations are against such an hypothesis. Thus, it seems hardly likely that such a delicate organism as the malarial parasite, which requires for its development not one but two distinct hosts, should be able to thrive indifferently in air, soil, or other media. Besides, wherever anopheles have been entirely suppressed malaria has almost vanished, which shows that the mosquitoes are apparently the sole carriers of infection from man to man. We may, therefore, conclude that turning up the soil and impure water play no etiological rôle in the production of malaria, except in so far as they offer facilities for the breeding of anopheles.

Prevention.—The measures for the prevention of malaria have for their object either the prevention of the infection of man by the mosquito, or the prevention of the infection of the mosquito by man. The principal methods are as follows:—

(i.) The destruction of the parasite in man by the systematic use of quinine. Koch has obtained marvellous results by this method, but it is scarcely applicable to large communities.

(ii.) The prevention of the access of the mosquito to man by the use of mosquito nets, or such means as sleeping in the upper storeys of houses, camp fires, etc.

(iii.) Extermination of the mosquito. Some good can be done by certain insecticides, such as chrysanthemum powder, sulphur, or kerosene oil, and

by abolition of their breeding places by means of proper drainage. The eggs, larvæ, or the adult insect may also be directly destroyed; but the method is too costly and laborious to be of much use.

A more efficient means for their suppression would be the pitting against them of some of their natural enemies. It is known that the larvæ of mosquitoes are swallowed by many aquatic insects, and the adults are killed off by bats, lizards, and dragon flies. It is not impossible that in the near future we may discover some plant that would prevent the breeding of the anopheles, or some innocuous animal which would destroy or displace this species.

Relation to Kala-Azár.—Kala-azár is a very fatal disease largely prevalent in Assam. The nature of this affection has not been sufficiently investigated, and conflicting views are held with regard to its etiology. According to Giles, it is the result of ankylostomiasis, either by itself, or in some cases complicated by a coincident malarial infection. Ross, on the contrary, regards it as an aggravated form of malaria, from which, however, it is distinguished by a rapid occurrence of cachexia, greater resistance to quinine, and by the fact that it frequently attacks several members of one family.

CHAPTER XXV.

DYSENTERY.

As is well known, a number of pathological lesions situated at the lower end of the large intestine are empirically included under the name of dysentery. The various types of the disease do not appear to have a common source of origin, and their etiology has not been satisfactorily worked out.

For our purpose, however, dysentery may be divided into the *bacillary* and *amœbic* varieties. The former has been ascribed by Celli to the *bacillus coli communis* taking on a virulent function when in the presence of the pyogenic cocci. It is also suggested that a mere catarrhal condition of the bowels (as induced by malaria, bad food, exposure, etc.) may enable the colon bacilli to become virulent and promote dysentery. But it seems difficult to imagine that so severe a disease as dysentery could be due to bacteria which are the normal constituents of the alimentary canal.

Shiga, in 1898, isolated an organism which he suggested as bearing a causal relation to the disease. It resembles in form and growth the typhoid rather than the colon bacillus. It is constantly present in the dejecta in acute cases, and gives the "agglutination reaction" when mixed with the serum of patients suffering from the disease. When introduced into the stomach of cats, characteristic

changes are produced in the lower intestine. It appears, therefore, that *Shiga's bacillus* is the undoubted cause of acute dysentery.

Amœbic Dysentery.—This variety is characterised by the presence of a definite species of amœba both at the seat of lesion and in the stools. If a piece of flocculent mucus be picked out from the fresh stools and examined under the microscope, the *amœba coli* can usually be observed. They throw out characteristic pseudo-podia, change their shape, and slowly move across the field

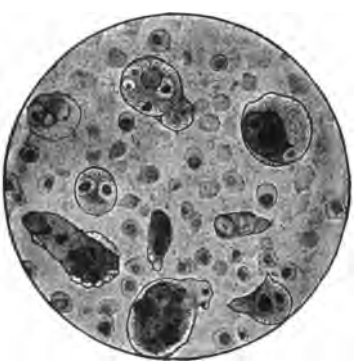


FIG. 26.—Amœba Coli.
[From Curtis's *Essentials of Practical Bacteriology.*]

of the microscope. When at rest the parasite is rounded in form and about four or five times the length of a red-blood corpuscle. There is a central nucleus, and the protoplasm frequently contains vacuoles, bacteria, red corpuscles, etc.

Outside the body, the amœba immediately breaks up, and cannot be made to grow on artificial media. Rectal injections of stools containing amœbæ give rise, in cats, to hæmorrhagic enteritis, the amœbæ being present in the

dejecta and frequently invading the mucous membrane of the intestine.

But these results cannot be regarded as conclusive. The amœbæ have not been used in pure cultures, and the results following the introduction of so complex a material as stools cannot, by any means, determine the properties of any single constituent. Then, again, the cat is an animal very susceptible to catarrhal inflammations of the bowels, which can be readily produced by the use of non-specific irritants.

However, the constant presence of the amœba in the submucous tissue, in the ulcers and in their spreading zone, cannot be regarded as an accidental coincidence. Further, the presence of amœbæ coli in the pus of liver abscess (a condition frequently associated with tropical dysentery), where they may exist without the pus cocci, shows that they have migrated into the portal circulation from the dysenteric ulcer and thence carried to the liver. This pus, moreover, when injected into animals, gives rise to lesions precisely similar to those produced by the injection of dysenteric stools. On the whole, then, we may conclude that a definite form of dysentery does exist in which the amœba coli plays an all-important etiological rôle.

CHAPTER XXVI.

IMMUNITY.

By "immunity" we mean a condition of the animal body, whereby it becomes resistant to one or more infectious diseases. The condition, indeed, is peculiar, for although enough pabulum is present in the tissues the infective agents cannot multiply and bring about the manifestations of disease. But it must not be imagined that immunity is ever absolute, and that bacterial growth and disease are invariably prevented; for it is always possible, by sufficiently increasing the dose and by lowering the vitality of the animal, to set up infection.

It may be asked, What is the reason of this immunity? What is the nature of the mechanism by which certain individuals escape the infection and others do not? As a matter of fact, every healthy person is provided with *protective* appliances in the shape of intact skin and mucous membranes, which usually suffice to resist the action of microbes. In addition to these we have the important influence of the lymphatic glands and of the gastric and salivary secretions. But, as the researches of Metschnikoff have fully established, the most important of all protective agencies is to be found in the leucocytes. When an infective agent invades the body, these cells, which normally patrol the blood-vessels and lymph spaces, become significantly increased in numbers. They

leave the vessel walls, move towards the foreign intruders, and engage them in mortal combat. They throw out protoplasmic processes and thus envelop and digest bacteria (*phagocytosis*). Apart from this phagocytic action, it is held that leucocytes also give rise to germicidal substances (*bacteriolysins* or *complements*), either by secretion or by disintegration. These are not, however, specific in their action, but are equally protective against all microbes.

The natural resistance of the tissues, however, cannot explain the immunity following non-fatal attacks of certain infectious diseases. In order to understand this question let us follow the course of an infective process, starting from the invasion and ending with the resulting immunity. We shall then be in a position to know the meaning of the incubation period, the manner in which disease is produced, how it ends in spontaneous recovery, and finally protects the patient from a second attack of the same disease.

Let us suppose then that, either owing to the lowered resistance or the greater strength of the attacking agents, the bacteria gain the upper hand and become settled in the tissues (tonsils, intestines, etc.). Here they immediately proceed to multiply and secrete toxic metabolic products. These enter into chemical combination with the constituents of the body cells and produce the characteristic symptoms of disease.

Ehrlich suggests that the protoplasmic molecule is a complex structure like other organic molecules, and comprises a nucleus of vital activity, to which are attached a group of "side chains" (*receptors*), capable of combining with various chemical bodies. When the toxic molecule combines with the receptors to produce poisonous symptoms a defect is created, the cell is damaged.

The cell now *reacts* in a characteristic manner, the aim of which is the destruction of the microbe and the neutralisation of its toxin. It throws out new side chains, which may not only cover the defect, but may actually over-compensate it. The excess of these side chains are cast off from the cell and constitute the *antitoxins*. The antitoxins circulate in the blood and intercept the toxins (forming neutral toxin-antitoxin compounds) before they have time to enter into combination with the tissue elements. No further symptoms can now occur, and the life of the individual is saved.

Side by side with these changes the bacteria themselves produce an antagonistic substance called the "*immune body*". The manner of its production is similar to that of the antitoxin. But, unlike the latter, it is insufficient to act alone—it must be combined with the ferment-like body (*complement*), already present in the serum, before the bacteria can be destroyed.

As soon as the toxins are neutralised and the infective agents destroyed, the disease is at an end. The patient recovers; but, owing to the previous stimulation by the toxins, his body cells have acquired a new property, and they continue to pour forth large supplies of antitoxins. These accumulate in the blood and protect him from a second attack of the same disease.

The individual enjoys this acquired immunity for a length of time which varies with different diseases. Finally, the protective substances are no longer produced, the immunity ceases, and the individual once more becomes susceptible to infection.

Immunity, which is acquired after a non-fatal attack of the disease or as the result of protective inoculations, is called "*active*," for it only follows after an active stimulation of the body cells, in the manner just described. It

is for this reason that "active immunity" is relatively stable and lasts for a considerable time.

This kind of protection must be carefully distinguished from "*passive*" immunity, which is observed after curative inoculations. It depends on the transference of the serum of an immunised animal to the body of another animal. The protective substances are thus introduced already prepared, and there is no attempt at the stimulation of cells. The immunity appears quickly but lasts only a short time, as the proteids introduced into the animal behave like a foreign body and are rapidly eliminated.

Immunised serum is "anti-toxic" or "anti-microbic," according as the toxin or the microbe is injected into the animal.[1] Thus, if the sub-lethal doses of an organism be repeatedly injected into an animal, the latter not only becomes highly resistant to the microbe, but its serum confers a similar immunity when transferred to another animal. Such sera are "*anti-microbic*" but not antitoxic, and are used in those diseases where the toxic effects are relatively small (cholera, plague, etc.).

To prepare *antitoxins* on a large scale, gradually increasing doses of the toxins are injected into a horse until

[1] If, instead of bacteria or toxins, other cells or cellular products (red-blood corpuscles, milk, ferments, nerve cells, etc.) be substituted, the corresponding antagonistic or "*anti-bodies*" may still be obtained. The mode of action of the anti-bodies varies with the nature of the substances producing them, and consists of such diverse manifestations as the neutralisation of toxins and ferments, the destruction and agglutination of cells, and precipitation. On account of their high specificity, anti-bodies have been largely used in the identification of the various substances producing them. Their interaction is so delicate as to enable us to distinguish the true from the false cholera germ, snake venom from other poisons, and the milk or blood of one species of animal from that of another. The scientific and medico-legal importance of these facts is self-evident.

it can tolerate enormous quantities of the same. The animal is then bled, the serum is mixed with some antiseptic, and passed through a Chamberland filter. Finally, the filtrate is "standardised"—*the "unit of antitoxin" being the amount required to neutralise the unit of toxin, i.e.,* 100 *times the lethal dose to a guinea-pig of* 500 *grammes* (Ehrlich).

APPENDIX A.

THE PRINCIPLES OF BACTERIOLOGICAL TECHNIQUE.[1]

In order to obtain a satisfactory knowledge of the biological characters of bacteria, it is necessary to obtain them in pure cultures by artificial cultivation, and next to subject them to a microscopical examination. For the purpose of testing their pathogenic power a further procedure is requisite, *i.e.*, experimental inoculation in animals.

I.—Cultivation of Bacteria.

1. *Sterilisation of Apparatus.*—All glass vessels, cotton wool, and metal instruments should be rendered germ-free by dry heat (150° C. for one hour). This is best done by means of special apparatus, but an efficient steriliser may be improvised out of an ordinary biscuit-box, and heat obtained from a convenient source. Another useful method is to wrap the apparatus in cotton wool and to apply heat until the wool is singed.

2. *Nutrient Media.*—Two of the most widely employed media are gelatine and agar-agar. Both are transparent and can be rendered solid or liquid at will. But while gelatine liquefies at a comparatively low temperature (25° C.), and so cannot be used in the tropics, agar-agar remains solid at blood-heat—a temperature which corresponds to

[1] For further details the reader is referred to Curtis's *Essentials of Practical Bacteriology*, and other works on the subject.

the optimum of most pathogenic bacteria. Many bacteria liquefy gelatine, but no organism has been known to liquefy agar-agar. For special purposes various substances are added to these media, *e.g.*, glycerine, glucose, lactose.

Other nutrient media commonly employed are blood serum, bouillon, milk, and potato.

A culture medium said to be very convenient in the tropical countries is the fluid contained in the interior of unripe cocoanuts. It contains glucose, vegetable albumen, and salts, and has the additional advantage of being contained in a germ-free receptacle.

After being prepared, all media are placed in flasks or test tubes, plugged with cotton wool, and sterilised by steam for three successive days (see p. 29).

3. *Inoculation of Media.*—This is conveniently done by means of a platinum needle, either straight or with a looped end and fastened to the extremity of a glass rod. The needle must be heated red-hot both before and after it is used. Similarly, the plug of the culture tube must be singed both before and after inoculation. Fluid culture media are inoculated by transferring a loopful of the material containing bacteria to the medium. When solid media are used a single "*stab*" is made with a straight needle, or, if the medium is solidified in a slanting position, the needle is simply "*stroked*" over the surface. Potato cultures are also inoculated by the latter method.

After inoculation gelatine tubes are kept at 20° C. to 22° C. but all others at 37° C. In order to maintain these constant temperatures "*incubators*" are frequently employed; but in the tropics a place in the verandah could easily be found where the temperature corresponded to 37° C. It is essential that the cultures which are being incubated should be kept *in the dark*, as light is inimical to the development of all microbes.

To isolate bacteria in *pure cultures* gelatine or agar plates are made. The latter medium is melted and next carefully cooled to a temperature not destructive to bacteria. The liquid medium is now inoculated, and rapidly poured into sterilised shallow glass dishes ("Petri dishes"), and allowed to solidify. These dishes, or, as they are now generally called, plates, are then incubated for two or three days, when colonies result from the growth of each bacterium. Finally, inoculations are made from one such colony to a tube of culture medium, and pure cultures of organisms obtained.

When numerous bacteria are present in the given material the necessary dilution may be made by inoculating a second tube from the first, and a third from the second. Plates are then made with the third tube, and the resulting colonies, being few in number, can be easily isolated.

II.—Microscopic Examination of Bacteria.[1]

In preparing specimens for the microscope it is essential that slides and cover glasses should be *absolutely clean* and free from the slightest trace of grease. They are best cleaned by soaking them in boiling nitric acid for ten minutes and then washing in water. They are subsequently kept in small closed jars containing alcohol.

1. *Hanging Drop Preparation.*—When it is desired to study the motility of an organism the method of cultivation in a drop of culture fluid attached to the under surface of a cover glass, and suspended over a hollow ground out of a glass slide, is very useful. A drop of culture fluid is transferred to the centre of a clean cover glass, which is then rapidly inverted over the hollow of the slide and fixed in

[1] An extremely useful bacteriological test-case, containing all the necessary stains and instruments required for this purpose, may be had from Mr. Martindale, New Cavendish Street, W.

position by a ring of vaseline. The organisms are most abundant at the edge of the hanging drop, where they must first be looked for.

2. *Cover Slip Preparation.*—In this method the material containing bacteria is spread out over a thin cover glass, dried, and stained for microscopical examination.

(*a*) *Preparing the Film.*—With a platinum loop heated to redness in the flame, a small drop of water is placed in the centre of the cleaned cover glass, and then, with the same instrument, a little material from the surface of a solid culture is taken with due precautions and mixed with a drop of water. In the case of liquid cultures the addition of water is evidently unnecessary.

The emulsion is next evenly spread over the cover slip and allowed to dry in the air. After the film is dry the cover glass is rapidly passed three times over the flame, so as to coagulate the albumen and *fix* the film to the cover slip.

(*b*) *Staining.*—This is effected by covering the film side of the cover glass with a stain, which is poured off after a few minutes, and the excess washed in water.

The penetrating power of stains is increased by heating or by adding to the stain carbolic acid, aniline oil shaken up with water, and other "*mordants*".

The dyes most commonly used are carbol-fuchsin, methylene blue, and aniline gentian violet. They are all kept in saturated alcoholic solutions in order to prevent decomposition. *It is absolutely necessary to filter the stains before use.*

Formulæ of Stains:—

(i.) *Carbol-fuchsin* is made by adding a saturated alcoholic solution of fuchsin to carbolic lotion (1 in 20) until the solution has lost its transparency.

(ii.) *Löffler's Methylene Blue* is prepared by adding to 100 c.c. of an aqueous solution of potassium hydrate (1 in 10,000) 30 c.c. of saturated alcoholic solution of methylene blue.

(iii.) *Aniline Gentian Violet.*—First prepare aniline water by shaking up into an emulsion 5 c.c. aniline oil and 100 c.c. distilled water. Then filter it through moistened filter paper.

To nine parts of the solution thus obtained add one part of concentrated alcoholic solution of gentian violet.

(iv.) *Eosin* is used in from 0·5 up to 5 per cent. watery solutions.

The average time required for staining may be given as follows: carbol-fuchsin, one to two minutes; methylene blue, five to ten minutes; aniline gentian violet, two to three minutes.

Sometimes the albuminous material in the film is so deeply stained as to obscure the proper view of bacteria. To obviate this, alcohol, mineral acids, and other decolorising agents are used. They clear up the background and render the outlines of bacteria more prominent. After decolorising, the background may be stained with a contrast stain such as eosin.

(*c*) *Mounting.*—When the staining is complete the preparation is washed, dried, and mounted in xylol balsam.

The following special methods of staining are most commonly used in bacteriological investigations:—

(1) *Gram's Method*:—

(*a*) Prepare a film preparation and fix in the usual way.

(*b*) Stain in aniline gentian violet for five minutes.

(*c*) Pour off excess of stain and treat with Gram's iodine solution (iodine 1 part, potassium iodide 2 parts, and distilled water 300 parts) for two minutes.

(*d*) Wash with alcohol until no more violet colour is discharged.

(*e*) Wash in water, dry and mount.

Gram's method is most useful for the differential diagnoses of pathogenic germs. Thus, the organisms of pneumonia, tubercle, leprosy, diphtheria, and tetanus are stained; whereas the organisms of cholera, plague, influenza, and typhoid fever are decolorised by this method.

(2) *Ziehl-Neelsen's Method of Staining the Tubercle Bacillus*:—

(*a*) Place fresh sputum in a glass dish against a black background, pick up a minute caseous particle, and spread it over a coverglass.

(*b*) Dry and fix the film.

(*c*) Float the coverglass upon the carbol-fuchsin solution and apply heat till steam commences to rise. The stain is allowed to remain on for five minutes afterwards. This stains everything on the film.

(*d*) Wash off the excess of stain in water.

(*e*) Decolorise by dipping in 25 per cent. sulphuric acid. This removes the stain from everything except the tubercle bacilli.

(*f*) Wash in water.

(*g*) Counterstain with methylene blue for two minutes. This stains non-acid-fast bacilli, leucocytes, etc.

(*h*) Wash, dry and mount.

In the case of tuberculous milk and urine, the fluids are first centrifugalised, and films are made from the sediment. The subsequent procedure is precisely as above described.

When a centrifugal machine is not available, carbolic acid is added to the fluid in amount sufficient to convert it into a 1 in 20 solution. This is allowed to stand for twenty-four hours, and then films are prepared from the deposit.

(3) *Staining of Spores.*—Spores can be stained by a method similar to that employed for staining the tubercle bacilli. After the film is prepared and carefully fixed, it is treated with 5 per cent. chromic acid in order to facilitate the penetration of the stain into the spore capsule. The stain is heated carbol-fuchsin; but the sulphuric acid should not be more than 5 per cent. The bacilli may be counterstained by methylene blue, the spores being stained red.

(4) *Staining of Flagella.*—The methods of staining flagella are somewhat complicated, but their essential features may be outlined as follows:—

(*a*) Fix the living bacilli by simple drying in the air.

(*b*) Add a mordant (*e.g.*, tannin and osmic acid). This forms a transparent film in which bacteria and their uninjured flagella are embedded.

(*c*) Wash off the excess in water.

(*d*) Add the stain (*e.g.*, silver nitrate).

(*e*) Wash, dry and mount.

(5) *The Demonstration of Malarial Parasite.*—Blood may be examined for malarial parasites either in the fresh unstained state or by means of stained preparations. If possible the examination should be made before the administration of quinine, as this drug causes the hæmamœba to disappear from the circulation.

A. *Fresh Preparations:*—

(*a*) Clean several coverglasses and slides.

(*b*) Cleanse the pulp of a finger tip with alcohol and then prick it with a clean needle. Wipe off the first drop of blood. Squeeze out from the puncture a second droplet and touch the apex of this lightly with the centre of a coverglass and place it on a slide. After a few seconds, when the blood has spread out in a fine film, apply vaseline

round the coverglass and examine with a $\frac{1}{12}$ inch immersion objective.

It is best to select a field in which the blood corpuscles are lying isolated and in a single layer. The interior of every red corpuscle should be carefully examined, and a negative diagnosis should only be made after a prolonged search.

B. *Permanent Preparations* :—

(*a*) Prick the finger and squeeze out a drop of blood as before.

(*b*) Apply a strip of cigarette paper (or the smooth edge of writing paper) to the blood at one end. Lay this (wet surface downwards) on a slide, and, after waiting a few seconds to allow the blood to spread over the slide, draw it horizontally along the glass. In this way a uniformly thin film is made in which the corpuscles are arranged in a single layer.

(*c*) Dry. Fix the film by immersing in absolute alcohol for five minutes and then dry again.

(*d*) Apply the stain (2 per cent. methylene blue, 5 per cent. borax in distilled water) for thirty to fifty seconds.

(*e*) Wash, dry and mount in xylol balsam.

All nuclei and parasites are stained blue. If required, the red corpuscles may be counterstained with eosin, in which case they are stained pink and contrast admirably with the blue colour of the parasites.

III. Experimental Inoculation of Animals.

The pathogenic power of bacteria can usually be demonstrated by injecting pure cultures into susceptible animals. The animals most suitable for this purpose are the rabbit, the guinea-pig, and the mouse. Great discretion should be

employed in interpreting the results, as some animals are naturally immune, while others modify the disease.

Inoculations are made with a sterilised hypodermic syringe into the subcutaneous tissue, or into the peritoneal cavity, or intravenously. The symptoms are observed during life and an examination made after death.

APPENDIX B.

SNAKE VENOM AND ANTI-VENOMOUS SERUM.

Two species of poisonous snakes are usually recognised—the viperines (viper, rattlesnake, etc.) and the colubrines (cobra, bungarus, etc.).

The venom, which is secreted by a gland in the region of the parotid, is a yellowish albuminous fluid, and in the dried state can be preserved for long periods. It contains two albuminous bodies, one of which acts locally on the tissues, and the other produces the general constitutional symptoms due to bulbar intoxication. By being heated to 85° C. for fifteen minutes the first-mentioned poison can be removed, when it is found that the venoms of different species produce the same effects, differing only in the degree of their activity. Although the poisonous substances are contained in venoms in different proportions, Calmette holds that the physiological action of the venoms of colubrines and viperines is, in essence, alike. Thus, the venom of the French viper is forty times less toxic than that of the Indian cobra, but the active substance of both varieties is essentially the same.

The mongoose and hedgehog can tolerate large doses of snake venom, but the immunity is not absolute, for they readily succumb when the dose is still further increased.

The so-called immunity enjoyed by the Indian snake-charmers is easily explained. In many cases the poison fangs are removed, but when these are intact the jugglers so familiarise themselves with the movements of the reptile

that they mostly escape being bitten. When the snake attempts to bite, the juggler looks at it with a strange fixed gaze and plays an instrument, so that it becomes spellbound by the gentle and plaintive cadence of the musician.

Professor Fraser has shown that normal bile possesses an antidotal effect against snake venom. This is probably due to a digestive power of the bile, and for its exhibition a certain interval of time is necessary. Thus, if a fatal dose of the venom is injected directly into the gall bladder of a rabbit, death supervenes in exactly the same time as when the injection is made in the subcutaneous tissue. Evidently in this case the venom is absorbed into the circulation before it has had time to be modified by the bile.

According to Calmette, antitetanic serum and normal bouillon have a preventive action similar to that possessed by the bile. These substances, therefore, do not act specifically, but merely cause a transitory stimulation of the leucocytes, which normally fix the venom and convey it to the central nervous system.

That the *leucocytes* play an important rôle in the fixation of venom is evident from the fact that the introduction of this poison into an organism is always accompanied by a hyperleucocytosis. Further, if we inject into a normal animal a fatal dose of venom, diluted in a fresh leucocytic exudation, we invariably observe a retardation in the poisonous symptoms, and very often the animal actually survives.

Antivenomous Serum or "*antivenine*" is derived from horses that have been immunised by gradually increasing doses of venoms obtained from the cobra and other poisonous snakes. The introduction of several kinds of venoms is necessary in order to obtain a serum which shall be equally protective against all species of serpents. Several authorities, however, deny this universal efficacy of Calmette's serum, and they urge that there should be a special

serum for each kind of snake. Such a suggestion is scarcely practicable, considering the difficulty and length of time (over fifteen months) involved in the preparation of any effective serum. Perhaps a satisfactory compromise would be to prepare different antisera for the colubrines and the viperines.

Lamb has recently asserted that antivenine does not keep well in India, but, on the other hand, Calmette affirms that flasks sent to Indo-China and India, and returned to him after eighteen months, have preserved their antitoxic properties "absolutely intact". It is necessary, however, to keep the bottle in a cool place and in the dark.

The ordinary dose of antivenine is 10 c.c. (*i.e.*, a whole bottle) for children and adults. But in the case of bites from the cobra and other highly poisonous snakes it is advisable to inject 20 c.c. (or even 30 c.c.), and to make the injection intravenously, instead of in the subcutaneous tissue. Intervention must take place immediately after the bite and should never be delayed beyond an hour.

Along with the serum, the application of a tight ligature close above the wound should never be forgotten. In order to neutralise any venom remaining in the wound, the latter should be bathed in a fresh solution of hypochlorite of lime (1 in 60) or in a solution of chloride of gold (1 per cent.).

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